"A STUDY OF HEPATITIS & SURFACE ANTIGEN IN PROFESSIONAL & VOLUNTARY BLOOD DONORS & ACUTE VIRAL HEPATITIS IN BUNDELKHAND REGION OF INDIA

THESIS

FOR

DOCTER OF MEDICINE

(MEDICINE)

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JHANSI

1990 VEER BAHADUR DHAKA

C E R T I F I C A T E

This is to certify that the research work entitled "A STUDY OF HEPATITIS B SURFACE ANTIGEN (HBsAg) IN PROFESSIONAL AND VOLUNTARY BLOOD DONORS AND ACUTE VIRAL HEPATITIS IN BUNDELKHAND REGION OF INDIA", which is being submitted as a thesis for M.D.(Medicine) Examination, 1990, of Bundelkhand University, has been carried out by Dr. Veer Bahadur Dhaka in the department of Medicine and Microbiology, M.L.B. Medical College, Jhansi.

He has put the necessary stay in the department as per university regulations.

Dated : 18.11.89

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Dated: 18.11.89

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A C K N O W L E D G E M E N T

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C O N T E N T

INTRODUCTION

Hepatitis B virus was discovered as a consequence of a population study designed to identify inherited immunologic traits in humans (Blumberg, 1977; Blumberg et al, 1984). Beginning in 1955, with the introduction of the starch gel electrophoresis method by Smithies (1955), it became clear that there were a large number of inherited biochemical polymorphism of human serum proteins. Allison and Blumberg (1961) postulated that if any of these polymorphisms, involved antigenic proteins, patients who received even a small number of transfusions would be likely to be exposed to proteins or other biochemical entities which they themselves had not inherited or acquired but which were present in the donors'blood. If this was to happen the transfused patient might develop a precipitating antibody against this "Foreign" protein and the antisera so formed could be used to detect the polymorphic protein system.

The above mentioned hypothesis was tested by using the serum from transfused patients as a reagent in agar gel immunodiffusion experiments (Allison and Blumberg, 1961). Blumberg and Colleagues (1961, and 1962) soon identified a previously unknown polymorphism of the serum low density lipoproteins (termed the Ag system) which has proved to be of interest in genetics,

diseases' studies, and forensic medicine. After the initial discovery the hypothesis that serum protein polymorphisms could be identified by the transfused patients technique continued to be tested and a new precipitin system that was distinctly different from the first was detected (Blumberg et al, 1964 and 1965). The initial reaction was between the serum of a transfused haemophilia patient from New York city and an Australian aborigine. The antigenic material present in the aborigine was termed "Australia Antigen" (Au) and a series of studies to determine the biologic significance of the unusual constituents were designed.

Further field studies were done to describe the distribution of the antigen in human population (Blumberg et al, 1965). From the studies, data were collected from which hypothesis could be generated. Australia antigen was very rare in the United States and northern European population (Prevalence about 0.1 per cent) but common in tropical and Asian groups (Prevalence 5-15 per cent) (Blumberg et al, 1965; 1966). Blumberg and colleagues (1965) also found Australia antigen to be common in patients with leukaemia, an observation which arouse as a consequence of systematic studies of the distribution of the Australia antigen in a variety of diseases.

From the Australia antigen - leukaemia disease association, the hypothesis was made that patients who were likely to develop leukaemia were also likely to have Australia antigen (Blumberg et al, 1967). A series of population with a high likelihood of developing leukaemia were identified and tested. These included persons with Down syndrome who were known to have much greater risk of developing leukaemia in childhood than the general population (Miller et al, 1964). The prediction was fulfilled in that more than 30 per cent of uninstitutionalized persons with Down syndrome were found to have Australia antigen in their blood (Blumberg et al, 1967 and 1968).

At about the time of this discovery (1966) a series of observations were made which directed attention to the possibility that Australia antigen might be associated with a hepatitis virus (Blumberg. 1977). The most important of these, in retrospect, was the case of JB, a patient with Down syndrome (Blumberg et al, 1984). In the original series of studies on people with Australia antigen, its presence or absence appeared to be a persistent characteristic (Blumberg et al, 1965, 1966). JB did not have Australia antigen in his serum in the initial analysis. Contrary to expectation, Australia antigen was found in his blood on subsequent examination. Concomitant with the appearance of Australia antigen, JB developed enzyme elevation and hepatitis was diagnosed on

liver biopsy. A hypothesis was made that Australia antigen was associated with a hepatitis virus. This hypothesis was tested with another series of epidemiologic studies (Blumberg, 1967; 1968 and 1969).

It was found that Australia antigen was more common in patients with acute and chronic hepatitis than in healthy people or patients with other diseases (Blumberg et al, 1967, 1968 and 1969). These observations were soon confirmed by Okochi and Murakami (1968) and later by others (Prince et al, 1968). From these population studies, a biologic model was formulated, namely that Australia antigen, was or was on, a virus which could cause hepatitis (Bayer et al, 1968). This model was tested by a series of experimental laboratory studies. Eventually the virus particle was isolated by column separation ultracentrifugation and enzymatic digestion of remaining serum proteins. It was visualised under the electron microscope (Bayer et al, 1968; 1970) and its transmission by transfusion (Goeke et al, 1969 and 1970) and animal inoculation was shown (London et al, 1970 and 1973). Thomas and Blumberg did not test hypothesis that Australia antigen was associated with a particular type of hepatitis (i.e. serum or infectious). Thomas and Blumberg approach was to perform cross sectional studies to elucidate the relationship of Australia antigen to the various clinical forms of hepatitis. Other workers were more direct. Prince (1968) reported the

presence of an antigen in serum(SH antigen) that he said was specific for serum hepatitis. He found serum hepatitis antigen in 6 of 8 cases of post-transfusion or postinoculation hepatitis during the pre-clinical or early clinical phase of disease, but not in the blood of five patients with infectious hepatitis. Shortly there after, Prince stated that serum hepatitis antigen and Australia antigen were identical (Prince, 1968).

An experimental approach was taken by Krugman and colleagues (1967). In order to learn more about the biology, etiology and prevention of viral hepatitis beginning in 1955, they induced hepatitis in children residing in an institution for the mentally retarded (Krugman et al, 1967). By 1968, they had distinguished two infectious sera, MS-1 and MS-2, collected during two episodes of hepatitis which occurred in a single patient. Inoculation of MS-1 regularly produced a short incubation disease (Krugman et al, 1967). Gile et al (1969) reported that MS-2 contained Australia antigen, MS-1 did not (Gile et al, 1969). Furthermore almost all children inoculated with MS-2 developed Australia antigen in their blood about the time of occurrence of hepatitis. None of the recipeints of MS-1 acquired Australia antigen. These studies demonstrated the specificity of the association of Australia antigen with one type of hepatitis and also strongly supported the hypothesis

that Australia antigen was part of the infectious agent which caused the disease.

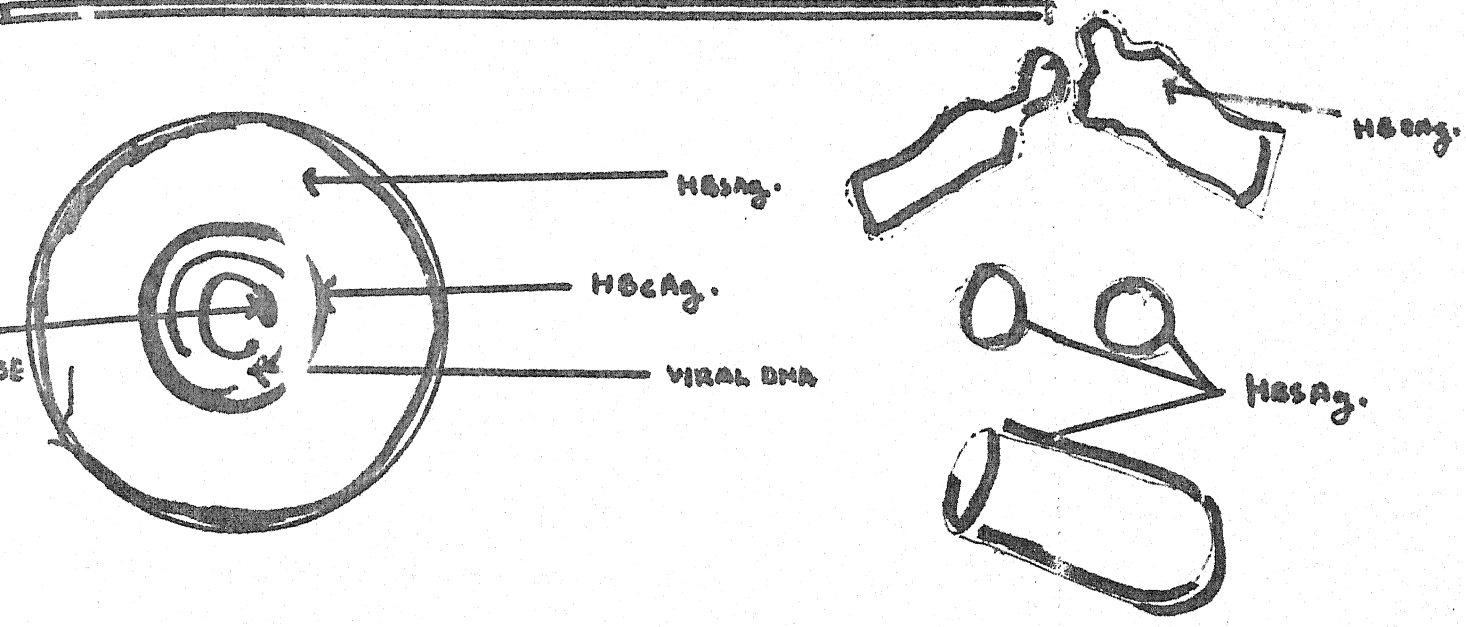
The importance of detection of Australia antigen in blood donor lies in the fact that some of the patients suffering from hepatitis B become chronic carriers, since they may be immunologically deficient. They are apparently normal individuals with Australia antigen in their sera without any clinical or biochemical abnormalities of liver functions. Hence the main significance lies in screening of professional and voluntary blood donors and reducing the incidence of direct transmission.

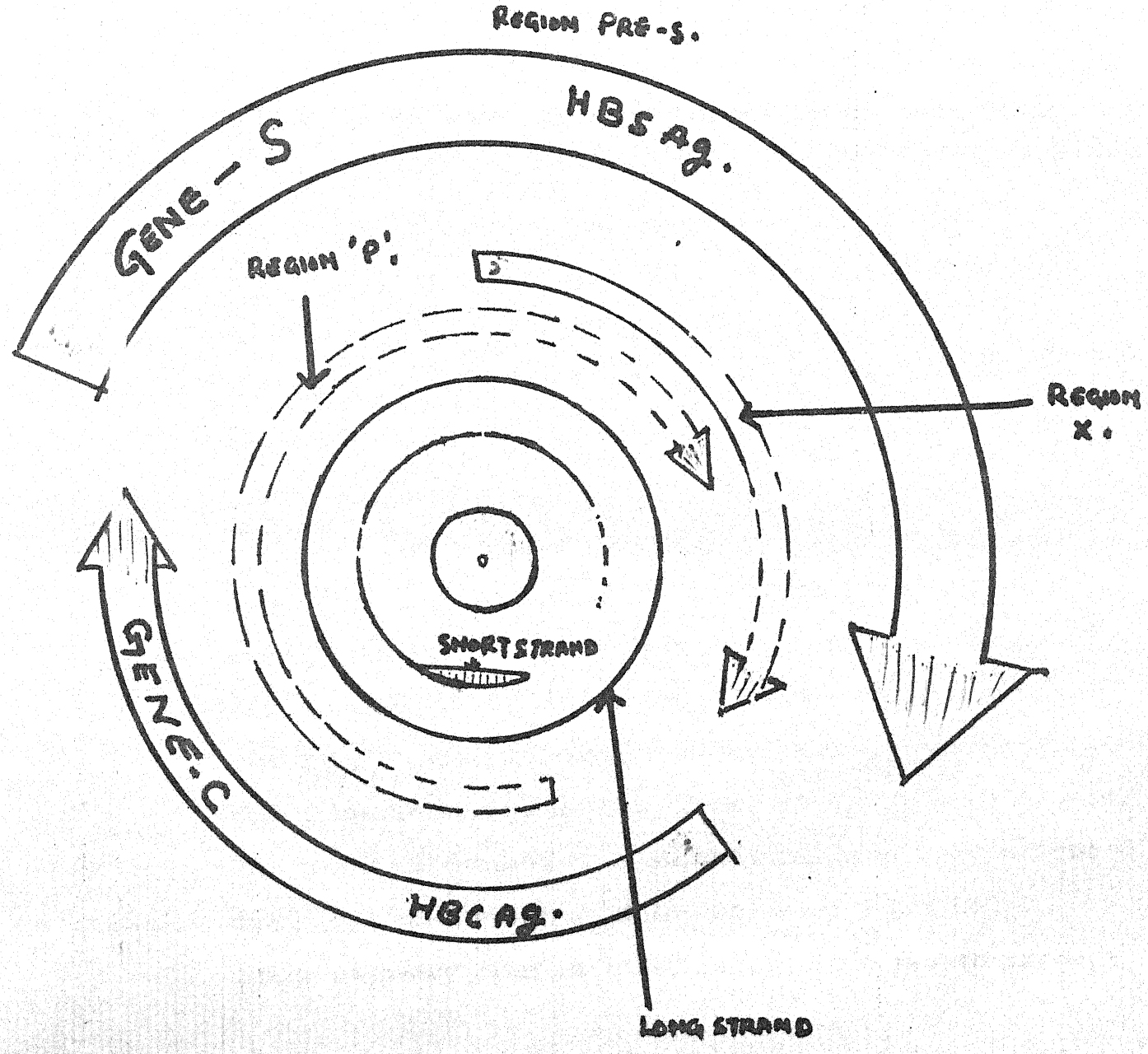
REVIEW OF LITERATURE

The morphologic structure, antigenic composition, DNA size and structure, DNA polymerase activity and biologic properties of HBV distinguish this agent from all previously known human viruses. DNA containing viruses which appear to be phylogenetically related to human HBV (Summer, 1981) as determined by ultrastructural criteria, circular DNA with large single strand regions, cohesive ends and approximately 3000-3300 base pairs, DNA polymerase activity circulating surface antigen particles and ability to induce persistent infection have been discovered in the eastern world chuck (Mornotanonax) (Summers et al, 1978; Werner et al, 1979). The California ground squirrel (Spernophilus buchevi) (Marian et al, 1980b) and the pekin duch (Anus domesticus) (Feitelson et al, 1981, Woodchuck hepatitis virus, infection is associated with the development of chronic hepatitis and hepatocellular carcinoma (Popper et al, 1981, Summers et al, 1978) and may serve as a key model in the study of the pathogenesis of other disorders which also are associated with persistent HBV infection in human beings.

Further study of this new class of HBV type virus may provide information about the biology of infection and virus host interactions which are critical to a complete understanding of human HBV. The virus of

HBV STRUCTURE





hepatitis B (HBV) is present in the sera of infected patients often at a very high level. Associated with it are three antigen antibody systems (Ag, Ab), the detection of which can be employed singly or in combination as markers for current or past infection. The presence of hepatitis B surface antigen (HBsAg)) denotes current infection and as such is the most useful single marker for identifying the potentially infectious patients. It is a complex antigen released from infected hepatocytes and exists in serum as three morphological forms. The bulk of serum HBsAg comprises excess virus coat antigen in the form of small 22nm particles and filament. The HBV particles (Dow Canison and Briggs, 1970) are 42 nm in diameter and have an outer coat of HBsAg. Inside this coat is a 27 nm core resembling a conventional small virus which carries with it the circular DNA genone and attendant DNA polymerase.

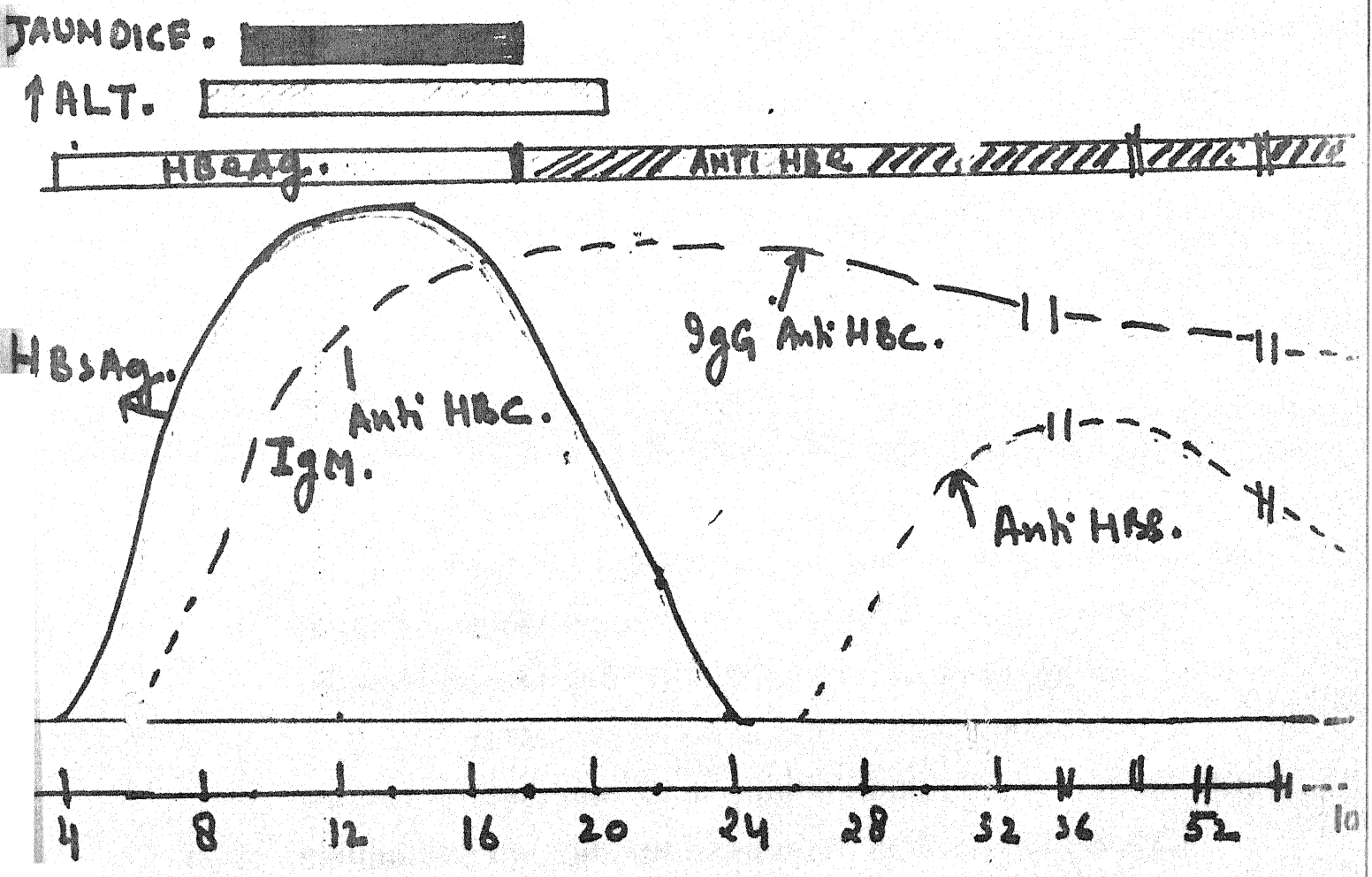
HBVAg carries a major antigen 'a' which is common to all HBV strains. In addition, two phenotypic antigens d or Y and W or 'r' are present (Le Bouvier, 1971), while these do not confirm any other particular attributes on the virus, they are useful for epidemiologists as they breed true. In other words, an a d r infection in a surgeon can only have arisen from an 'adr' source. This can be of great help. After recovery from an infection, most patients produce

antibody to HBsAg, anti HBs, the specificity of which is directed mostly to the common 'a' determinant. In some patients it may be months or some times even longer before serum anti-HBs can be detected. Anti-HBs denotes past infection and immunity to further infection.

The 27 nm nucleocapsid (core) of the human HBV contains the viral genome; circular, double standard DNA, comprising about 3200 base pairs, with a large but variable single standard region, and specific discontinuities or nicks which confer cohesive ends on the molecule (Sattler and Robinson, 1979). In studies of a human hepatocellular carcinoma cell line (PLC/PRF/5) which produces HBsAg and in which produces HBsAg and in which HBV genomes are integrated into host DNA, it has been suggested that integration occurs at the nicked cohesive end region (Edman et al, 1980). A protein molecule is covalently bound to the 5' end of the complete DNA strand but its function is uncertain (Gurlich and Robinson, 1980). The DNA polymerase associated with the HBV nucleocapsid is believed to repair the gap in the short DNA strand by lengthening its 3' end. In addition to HBV specific DNA polymerase, the nucleocapsid of HBV is also associated with the protein kinase activity (Albin and Robinson, 1980). The kinase serves to phosphorylate the major polypeptide of the core.

TYPICAL CLINICAL AND LABORATORY FEATURES OF

ACUTE VIRAL HEPATITIS TYPE B.



Cloning of the HBV genome, a major technological advance in the study of HBV, has been reported by several laboratories (Borrel et al, 1979; Charnay et al, 1979; Sninsky et al, 1979; Valenzuela et al, 1979). Restriction endonuclease cleavage maps of the clonedHBV genome has been constructed and the nucleotide sequences have been established.

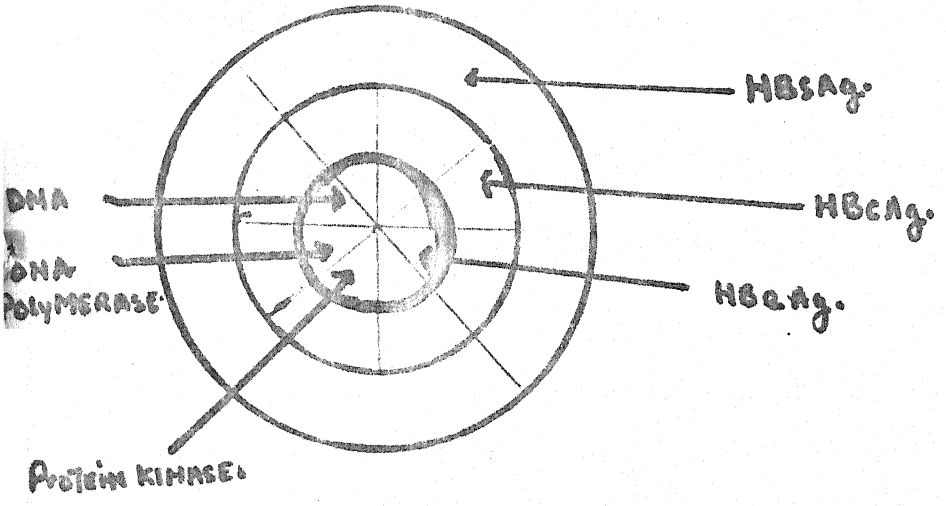
Different antigenic subtypes of HBV have yielded distinct restriction endonuclease cleavage sites for cloned DNA (Price et al, 1980), but the restriction patterns of clones derived from the same source are similar (Swinsky et al, 1979). These observations suggest homgeneity of the HBV genome in any given infected individual. Regions of the genome coding for the major polypeptides of the surface and core antigens of HBV have been identified appear to be present on the same DNA molecule, and recombinant plasmids which direct the synthesis of these peptides have been developed (Edman et al, 1981). RNA transcripts specific for the surface antigen sequencies of HBV, DNA has been demonstrated (Adman et al, 1988). A detailed review of present knowledge of the genetic organisation of HBV is available (Tiollais et al, 1981).

Following the successful cloning of HBV in bacterial plasmids, it became feasible to introduce the clones HBV DNA or its fragments into mammalian cell lines (Hirschman et al, 1980); Morurty et al, 1981). These models are likely to provide information about

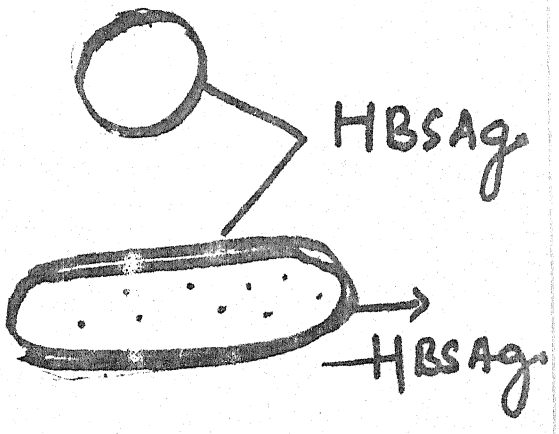
mechanism of intracellular HBV infection and replication which cannot be achieved by further characterisation of HBV DNA per se. The Hela cell Model is of particular interest (Hirschman et al. 1980). When Hela cells were exposed to cloned HBV DNA which had been excised from a plasmid and recircularised, they showed cytopathic changes, and, 11-14 days after subculture, produced both the surface and the core antigen of HBV. Further more, 42 nm HBV like particles, as well as smaller, 20 nm particles resembling surface antigen, particles were detected in the culture media of the Hela cells transfected with cloned HBV DNA. These data suggest that this widely available cell line is suitable for studies of the production in intact HBV as well as synthesis of its gene products (Raymond S Koff, 1983).

Another approach to further understanding of the biology of HBV has been the establishment of the tissue culture cell line (PLC/PRF/5) derived from hepatocellular carcinoma in a mozambican carrier of the hepatitis B surface Ag. This hepatocellular carcinoma cell line shows typical epithelial cell growth and retains the ability to produce surface antigen which is identical to that isolated from HBV carriers (Skelly et al. 1979). The cells exhibit many of the functions of hepatocytes, e.g. they secrete liver specific proteins such as alpha fetoprotein and form solid tumours when transplanted into athymic Nude Mice (Bassendine et al. 1980). Although

COMPANENTS OF THE HEPATITIS B VIRUS.

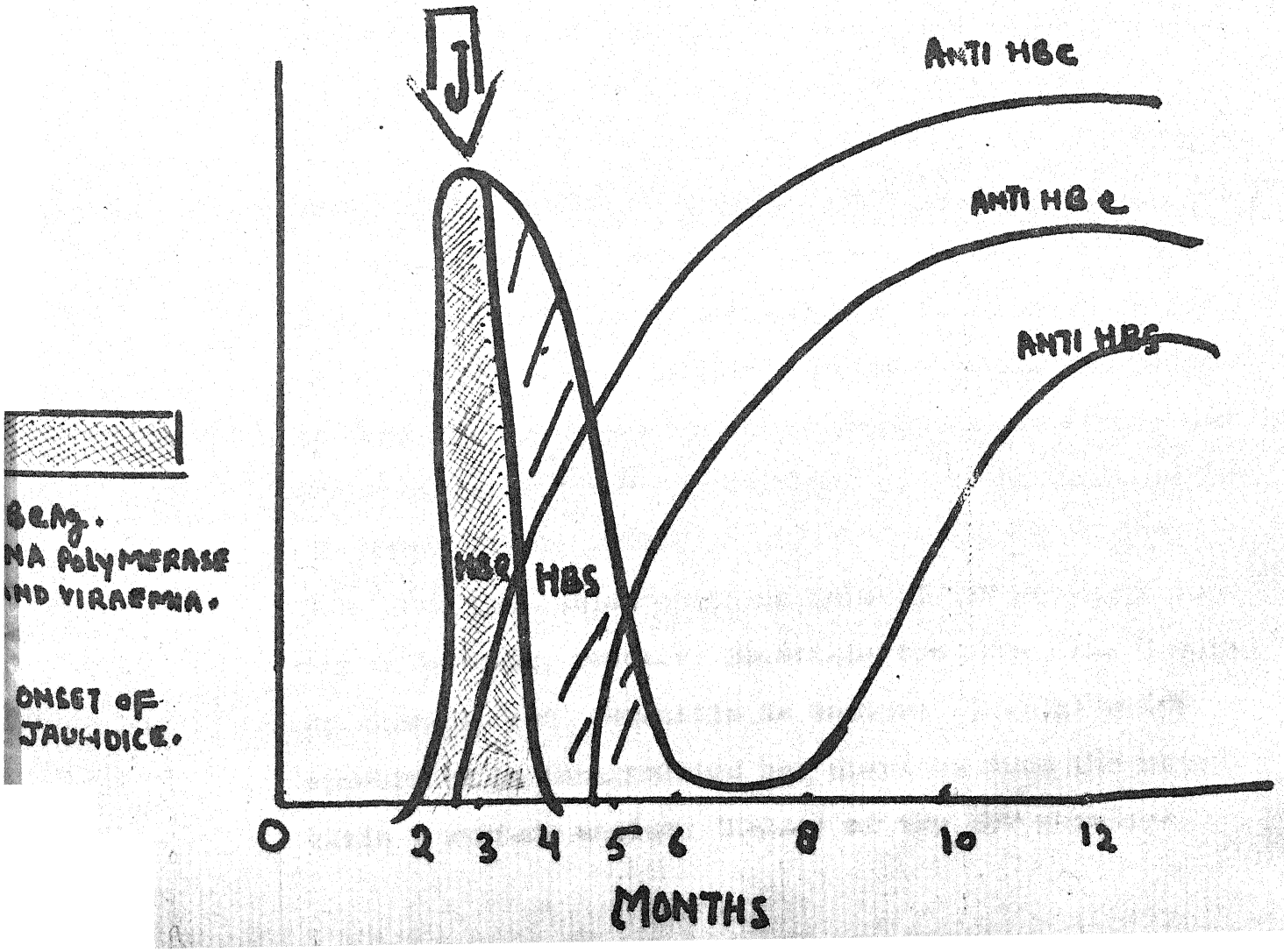


VIRION OF HBV.



SURFACE ANTIGEN PARTICLES.

THE ANTIGEN AND ANTIBODY MARKERS OF HEPATITIS B.



PLC/PRF/5 cells do not produce the core of Ag of HBV or intact infectious HBV particles (Daemer et al, 1980), and only RNA transcripts specific for surface Ag have been detected (Edman et al, 1980), molecular hybridization with cloned HBV DNA probes indicate the presence of complete or nearly complete sequences of HBV DNA (Brechot et al, 1980); Chakraborty et al, 1980; Marion et al, 1980 'a'). Restriction fragment hybridization studies indicated that most, if not all, of the viral DNA in infected cells is integrated into host DNA (Brechot et al, 1980; Chakraborty et al, 1980; Marion et al, 1980). Several copies (3-4) of HBV DNA are present per haploid cell DNA equivalent and 3-6 specific integration sites have been identified. Other cell lines, also derived from human hepatocellular carcinomas, and which secrete the surface antigen of HBV have been established (Knowles et al,1980) and provides experimental models for further investigation. These exciting techniques may be crucial to the search for knowledge of the mechanism underlying persistent production of HBV products following infection and to understanding the oncogenicity of HBV in human beings (Raymonds Koff, 1983).

The nucleocopsid or core of HBV contains two immunologically reactive materials the hepatitis B core Ag (HBsAg), and Hepatitis Be antigen (HBeAg) which appears to be interrelated but distinct from the hepatitis B surface antigen (HBsAg) of the HBV envelope.

HBcAg and HBeAg have been detected in the nucleus of hepatocytes of patients with persistent HBV infection (Yoshi-zawa et al, 1979). HBcAg is also present in the nucleus of acutely infected patients. It was detected in liver biopsy specimens in 3 of 24 patients with acute hepatitis B (Mathisen et al, 1973). Six of these 24, had HBsAg on the surface of their hepatocytes. HBcAg is not readily detected in the serum of infected patients because antibody (HBcAg) is invariably present and marks the presence of HBcAg. However, separation of anti HBc from HBV particles permits identification of HBcAg in the sera of the patients with persistent HBV infection (Rezzetto et al, 1981). HBeAg has been identified in HBsAg positive sera exclusively although there is one, unconfirmed report of HBeAg positivity in the absence of HBsAg (Tabor et al, 1980b).

A variable number of constituent polypeptides have been isolated from HBV cores. HBcAg is believed to comprise a major polypeptide weighing 19000 daltons with 183-185 amino acids as determined by the Nucleotide sequences of the HBV genome coding for its production. (Tiollais et al, 1981). HBeAg can be released from the inside of HBV cores. after treatment with detergent Sodium Dodecyl sulphate (Taka hashi et al, 1979). Two polypeptides with weight of 19000-45000 daltons were identified, Both had HBeAg activity. In other studies degradation of core particles resulted in the antigen

conversion of HBcAg to HBeAg (Ohori et al, 1980). These studies suggested that the antigenic determinants of HBcAg and HBeAg reside on different protein structure of the same 19000 dalton polypeptide. Whether this polypeptide is immunogenic and can raise antibodies against HBcAg and HBeAg remains to be determined (Raymond S. Koff, 1983). The relationship of the 19000 dalton polypeptide to the three antigenic components of HBeAg now recognised (Murphy et al, 1978), also remain uncertain (Blancy et al, 1980).

All individuals acutely infected with HBV become HBeAg positive. HBeAg in serum is detected shortly after the appearance of HBsAg and in most instances, disappears while HBsAg is still present (Krugman et al, 1979). In persistently infected HBsAg carriers, prevalence of HBcAg is variable but independent of sex, race, and HBsAg antigenic sub-type (Szmuness et al, 1981a), since a steadily increasing proportion of carriers clear HBeAg with time, older carriers are less likely to be HBeAg positive than younger once. The presence of HBeAg appears to incidate active viral replication, an abundance of intact HBV particles, and an increased risk of infectiousness in number of epidemiological settings (Porrillo et al, 1979). It remains an excellent, if imperfect markers of potential infectivity and may have clinical value in assessment of patient with HBV associated chronic hepatitis (Hoofnagle et al, 1981). In

addition, HBcAg may play a role in immunopathogenesis of the immune complex mediated membranous glomerulonephritis associated with HBV infections (Ito et al, 1981).

HBsAg is the most extensively studied of the known antigen of HBV. Infected hepatocyte synthesize HBsAg in excess of that required for the envelope of HBV large number of 20-22 nm HBsAg particles appear in the circulation and HBsAg is detectable on the surface of hepatocytes. The small, non infectious HBsAg, particles are immunogenic and because they induce antibodies which are protective, they have served as the source for an effective, safe, commercially available vaccine against HBV (Szmuness et al, 1981b). Biochemical characterization of HBsAg has disclosed the presence of number of polypeptides, some of which are glycosylated (Shiraishi et al, 1980). Although isolated polypeptide have varied in size, major component have molecular weight of 22000-23000 and 27000 daltons; different degrees of aggregation and glycosylation may be responsible for the observed heterogeneity. Solubilization of partially purified 22 nm HBsAg particles in sodium dadecyl sulphate, in the absence of reducing agents, yielded a polypeptide measuring 49000 daltons (Mishrro et al, 1980). This polypeptide was split, on incubation in reducing conditions, into two polypeptides with molecular weight identical to that of major polypeptides. The 49000 daltons peptide was immunogenic and

retained the common and sub type determinants of the source HBsAg particles (Raymond S Koff, 1983).

From neucleotide sequencing studies, an amino acid sequence of 226 residues has been suggested for one of the major HBsAg polypeptides. Chemically synthesized peptide corresponding to these amino acid sequences have been prepared, they have been shown to elicit antiboties to both native HBsAg and the major HBsAg polypeptides (Lerner et al, 1981).

Antigenic sub determinants of HBsAg coded for by the genome HBV are useful as epidemiological tools in tracing infection and serves as markers of population migration patterns. The hepatitis B vaccine prepared from HBsAg subtype ad particles confers cross protection (Szmuness et al, 1981b). The rare occurrence of HBsAg of different subtypes in the same individual may reflect double infection by different HBV's or exchange of DNA sequences between HBV of different subtypes (Hess et al, 1979).

The association of human serum proteins and purified preparation of HBsAg has been extensively studied. A species specific receptor for polymerized human albumin has been demonstrated on HBsAg particles (Imai et al, 1979), on individual HBsAg polypeptides (Ionescu - Matin et al, 1980), in the cytoplasm of HBsAg containing hepatocyte, 'Thung and Garber, 1981a), in PLC/PRF/5 cells and their media in tissue culture,

(Thung and Garber, 1981a), and on HBsAg particles produced by these cells (Ionescu-Matiu et al, 1980). The association of polymerized human albumin with the surface of hepatocyte and the receptor on HBsAg of the intact HBV particle may be a important determinant in the attachment of HBV to hepatocytes. However, the precise role of this system and its interaction with antibodies to polymerized human albumin (Hung and Gerbar et al, 1981b), and the complement sub component, C1q (Milich et al, 1981), are the subjects of continuing investigation.

There is wide global variation in the prevalence of the carrier. In UK blood donors it is between 0.1 and 0.2 percent. It ranges from about 5 percent in mediterraneah countries to as high as 10% or more in the countries of south east Asia (Sobaslavsky, 1978). One consequence of the high prevalence of carriers in those countries is the high incidence of chronic liver disease, cirrhosis and hepatoma. The higher prevalence of severe liver disease amongst carriers in many mediterranean countries contrast with the relatively benign course of HBsAg carriage in Britain and may be due to the Co-passage in these countries of another hepatic virus like agent - The delta agent (Rizzetto et al, 1977).

The carrier state is not static, it evolves with time and, at the outset, the markers present in the serum will (except for the absence of anti HBcAgm) be very similar to those of an early acute infection.

However, unlike the acute infection, where events occur with a time course measurable in weeks or months, the carrier state evolves over years or decades (Tedder,1983).

It is possible to divide carriers broadly into two groups - on the basis of their HBcAg/Ant& HBe status. Early in the carrier state the patient will have the circulating HBeAg. Serum from such patients is known to be infectious where small volume inoculation accidents have occurred (Alter et al, 1976). These patients also have high level of circulating HBsAg, DNA polymerase and tend to have mildly elevated serum transaminases (Barbora et al, 1978). They are known to be infectious for perinatal (Okada et al, 1976) and horizontal (Shikata et al, 1977). Transmission some time after the beginning of the carrier state, the patient will sero convert from HBeAg to anti HBe. It will usually takes years for this to occur (Miyakawa and Mayumi, 1978) although it may some time happen after a few months.

The detla agent antigen is distinct from the known antigens of the nucleocapsid and surface of HBV, and antibody to delta antigen is unrelated to known antibodies induced by HBV infection. The delta antigen was initially detected by immunofluorescent techniques in the nucleus of hepatocyte of patients with persistent HBV infection (Canese et al, 1979). Antibody to delta antigen could be detected in the sera of HBsAg carriers in the various parts of the world, although the

prevalence is highest in Italy (Rizzetto et al, 1979). The delta antigen has been partially characterized as a 68000 dalton protein (Rizzetto et al, 1980a) which in the serum of infected chimpanzees and humans is associated as an internal component, with a 35 to 37 nm subpopulation of HBsAg particles and a RNA molecules smaller than that of known RNA viruses (Bonino et al, 1981; Rizzetto et al, 1980c). Delta agent is transmissible to chimpanzees (Rizzetto et al, 1980b). Because delta results in chronic infections only in the presence of persistent HBsAg and may be transmitted by superinfection or co-infection of HBsAg carriers, it is believed to be a defective pathogenic agent requiring the helper function of HBV replication for its synthesis. The pathogenecity of infection with the delta agent is still incompletely understood but appears to be inversely related to the extent of productive HBV Replication; HBsAg carriers with diminished HBV synthesis appears to be an increased risk of developing chronic delta infection (Smedele et al, 1981).

In addition to its presence in blood, HBsAg in blood vessel walls has been identified in body fluids and secretions in glomeruli, but not in the faeces of HBV infected individuals (Feinman et al, 1979). Intact infectious HBV particles are present in the liver and blood and also have been demonstrated, by experimental transmission to honhuman primates in semen and saliva

(Scott et al, 1980). Whether HBsAg and HBV are simply deposited from the circulation or reflect HBV replication in the testes or salivary glands is unknown. The presence of HBsAg in bile is consistent with the notion of hepatic replication of HBV but replication of HBV in extrahepatic sites has yet to be established (Raymond S. Koff, 1983). The presence of HBsAg in pure pancreatic juice (Hoefs et al, 1980) and the identification of both HBsAg and HBcAg in the cytoplasm of pancreatic acinar cells is intriguing (Shimoda et al, 1981).

HBsAg may be detected in blood as early as six days after parenteral exposure to HBV (Krugman et al, 1979) in most infected patients HBsAg appears considerably later (1 to 3 months) but usually before the onset of biochemical evidence of hepatic injury. Blood obtained during the incubation period, before either HBsAg becomes detectable or serum aminotransferase levels are elevated has been shown to be infectious (Rinker and Galambos, 1981). The detection of HBeAg, by a widely available radioimmuno essay (Mushahwar et al, 1981), does not add to the diagnostic specificity of HBsAg. Antibody to HBeAg (Anti-HBe) appears immediately after HBeAg disappears and may remain detectable for a protracted period (Krugman et al, 1979). Mushahwar et al, 1981). Early in the course of HBV infection, concomitantly with or near the time of appearance of HBsAg, all patients develop anti HBc. It may be the only detectable marker in the period between the

disappearance of HBsAg and appearance of measurable antibody to HBsAg (Anti HBs) (Raymond Sl Koff, 1983).

The combination of tests for HBsAg and anti HBc permits the detection, without exception, of all acute HBV infections but does not distinguish between acute and chronic infection. Anti HBc may be detectable for years following acute infection (Krugman et al, 1979), and is present in HBsAg carriers with or without chronic hepatitis. High titres of IgM anti HBc are almost invariably present in acute HBV infection and may persist for several months to 1 to 2 years, and occasionally longer, after clearance of HBsAg (Kryger et al, 1981; Lemon et al, 1981). IgM anti HBc is also present in many HBsAg carriers but in lower titres than following acute infection (Kryger et al, 1981). Quantitative assessment of IgM anti HBc may help in distinguishing between patients with acute hepatitis B and HBsAg carriers with acute Non A, Non B hepatitis. IgM anti HBc in carriers is believed to indicate continuing HBV infection it may be useful in identifying low level carriers who are infectious (Raymond S. Koff, 1983).

Since secreening of the blood and blood products for HBsAg has been widely adopted the classical mode of blood borne transmission i.e. transfusion, is now responsible for only a minor fraction of HBV infections. In high risk regions, maternal infant and intrafamilial spread appears to be responsible for perpetuation of HBV infection and its association with the development chronic liver disease and primary hepatocellular carcinoma(Beasley et al,

1981a).

Maternal infant transmission involves carrier women, women with acute hepatitis B in the third trimester and mothers with acute hepatitis B during the first five weeks after delivery (Tong et al, 1981). The high frequency of HBV transmission, when acute hepatitis B occurs in the early post partum period suggests that close contact between mother and infant may play a more important role than previously believed. The precise mode of transmission is ill defined. Oral secretions, e.g. saliva, which may contain intact HBV particles (Scott et al, 1980), appear to have a low infectivity potential but may play a role under intense exposure conditions. The exachange of vehicles contaminated with oral secretions may be responsible for intra-familial clusters of HBV infection among children (Leichtner et al, 1981). Similarly, transmission of HBV in class rooms, from carrier children to classmates (Oleske et al, 1980), may involve physical transfer of virus to oral mucosal surfaces. The frequency of spread of HBV in schools may depend on the numbers of carriers and susceptibles and their class room behaviours (Raymond S. Koff, 1983).

The presence of HBV in semen (Scott et al, 1980) and HBsAg in menstrual discharge (Inaba et al, 1979) and the documentation of transmission between spouses (Inaba et al, 1979; Perrillo et al, 1979), support a venereal mode of transmission. The extra ordinary high risk of HBV infection in homosexual men, confirmed in the hepatitis B

vaccine studies (Szmuners et al, 1980b), indicates remarkable efficiency of intimate sexual contact in transmitting HBV. The total prevalence of HBV markers, in homosexual, was 68% in base line studies (Raymonds Koff et al, 1983) designed to identify susceptibles. During the two year follow up period 26% of the susceptibles who received placebo developed evidence of HBV infection (Szmuners et al, 1981b). Inoculation with shared contaminated needles may lead to HBV infection in illicit drug users and accidental percutaneous inoculation with contaminated needles is responsible for some instances of Nosocomial hepatitis B. Contamination of environmental surfaces with infected blood (Lauer et al, 1979), may contribute to the occurrence of HBV transmission in clinical laboratories and haemodialysis units in which exposure to blood is common place. Transmission of HBV by infected health care workers to their patients is infrequent but transfer of HBV from minor laceration and inapparent breaks in the integrity of the skin of the hands may be responsible for out breaks involving infectious oral surgeons and densits (Hadler et al, 1981).

The notion that haematophagous arthropods play a role in HBV transmission is supported by the persistence of HBsAg in bed bugs as long as six weeks after a single meal of HBsAg positive blood (Ogston et al, 1979). Epidemiological evidence for orthropod home transmission is absent. Similarly faecal oral spread and our breaks

of hepatitis B due to ingestion of faecally contaminated food and water not documented(Raymond S Koff et al,1983).

Shiff (1975) and Zuckerman (1978) have reported that following HBV infection, HBsAg usually disappear within 9 to 12 weeks. All cases with complications following HBV infection are associated with persistent antigenemia though a vast majority of carriers are asymp tomatic. Antigenaemia is also common in patients with leukaemia, leprosy, down syndrome, Hodgkin's disease, chronic renal failure on dialysis and in I/V drug addicts. Hepatitis B infection in males and during childhood is also followed by a higher carriers rate(Zuckerman, 1979).

The stimated number of HBsAg carriers in the world is ±20 to 125 million(Zuckerman, 1978). One to two percent of voluntary blood donors in USA are HBsAg carriers. In tropical countries the carrier states is much more common upto 20% being reported by Zuckerman,(1978).

In India the incidence of carrier state of Australia antigen in healthy adult male and female volun- tary blood donors has been reported to be 2.2% and 1.2% respectively (Pal et al, 1973). High incidence of antigen in blood donors has also been reported by other workers from different parts of the country (Sehgal and Aikat, 1970; and Dutta and Mohammad, 1972).

Blumberg et al (1968) reported 2.3% positivity of HBsAg among 127 serum samples of south Indian origin by immuno-electro-osmophoresis (IEOP). There is no

remarkable difference of carrier rates amongst healthy population of North and Sourth India. The prevalence of HBsAg is definitely higher in Indians than in Americans (0.1 to 0.2%) and Denis population (Gocke et al, 1969; Bunke et al, 1971). In tropical countries the incidence is 0.2 to 0.5% and in South east Asia as high as 20% in certain endemic areas (Blumberg et al, 1970). In Greek population the incidence is 0.8 to 1.8 percent. which is similar to that in India (Blumberg, et al, 1970).

In a study conducted on pregnant women attending the antenatal clinic for routine check up at Trivandrum 2.6% of them had Australia antigen (Shanmugam and Raj-Sekharan, 1982), whereas in pregnant woman of Kerala an incidence of 3% was recognised; the cord sera were also found to be positive in 1.7%. For Australia antigen in Bombay (Shanmugam and Raj-Shekharan, 1982). In Lahore 9.3% of the women were reported to be HBsAg positive during pregnancy. They are carriers and continuous to show the same antigenemic state, until 6 months or more after delivery. The persistent carrier rates of HBsAg among symptomatic mothers was found to be very high ranging from 66.1% to 100% in Finland, UK, USA and Senegal (Szmuness et al, 1973; Tapp et al, 1976 and Ukkohen, 1980).

Recently Derso et al (1978) reported that 8% of children of HBsAg carrier mothers from Asian communities (India, Pakistan, Bangla Desh) are HBsAg carriers.

The risk of serum hepatitis from blood transfusions, varies from 10 to 70 fold depending upon the source of blood (Allen, 1972). The hepatitis which followed HBsAg positive blood transfusion also have been reported to be severe (Cocke and Kavey, 1969). Prior experience of frequency of HBsAg in blood donors of New Delhi was 2.73% in 1973 (Shama et al, 1973) which increased to 3.9% in 1975 (Pastakia et al, 1975). Incidence of Australia antigen increased at alarming rate until 1970 in large cities mostly because of practice of needle sharing by parental drug users. The sale of blood by indigents to commercial blood banks had resulted in increased infectivity to those who received commercially prepared blood products (Ashcavai and Peters, 1971).

In some cities the incidence of clinical hepatitis acquired from a blood transfusion had been as high as 20 per 1000 units of blood. Although two thirds of patients contracting hepatitis had developed subclinical disease only (Grady, 1970; Taswell et al, 1970 and Allen et al, 1972). Pooling of blood products obviously increase the risk of transmitting the agent and storage of plasma for 6 months at room temperature is not effective in completely inactivating the agents (Redekar et al, 1968).

Blood fibrinogen carries a particularly high risk of transmitting hepatitis B(Boeve et al, 1969).

Viral hepatitis type B has a world wide distribution and transmission takes place by parenteral

infections. The virus is also present in saliva (Brodersen et al, 1974; Plainson et al, 1975 and Villiarezos et al, 1974). Tears, ascitic fluid sneez droplets and blood sucking insect (Zebe et al, 1972; Prince et al, 1972) and rarely in urine (Villiarezos et al, 1974) menstrual fluid (Darani and Gerbar, 1974) involved kissing (Villarezos et al, 1974 and Prince et al, 1972), bitting (Macquarri et al, 1974) razors and tooth brush (Mahley, 1975).

Study from different parts of the world (Blumberg et al, 1968; Okochi Murakami, 1968; Wright et al 1969 and Fox et al, 1969) have high lighted the difference in the incidence of Australia antigen in viral hepatitis. 50% of acute hepatitis in the USA is caused by HBV (Australia antigen) (Purcell et al, 1978), 23.6% of cases endemic hepatitis in India are HBsAg positive(Joshi et al, 1977 and Dutta et al, 1977).

The incidence of HBsAg in acute and non epidemic viral hepatitis has been widely variable in India. An incidence of 44% was reported from North India(Sehgal and Aikat, 1970), 22.6% from Delhi Cantth (Dutta and Mohammed, 1972), 22% in Patient admitted to naval hospital, Bombay (Gupta, 1973), 23% at Military Hospital Chandigarh and 10% in Non fulminant hepatitis at Chandigarh (Pal et al, 1973).

Western workers reported higher incidence (Gocke and Kavey, 1969) with 83% positivity within x

12 days of infection. Ringert (1971) reported 100% positivity rate in such cases within 10 days of infection and 53% after 20 days of infection, whereas an incidence of 40% was recorded by Nelson et al (1971) and 33% by Smith et al, (1973). There is variation in positivity in tubercular and non tubercular patients; Tubercular patients had 44.62% positivity within 8 to 14 days of hepatitis.

Sixty five to seventy five per cent of mothers who have hepatitis B, in their late pregnancy or during the immediate postpartum period transmit HBsAg to their children. Whereas the rate of transmission is only 3.5 to 5 percent in asymptomatic carriers. Severity of hepatitis transmitted to offsprings is greater when mother is asymptomatic (Wands et al, 1979).

Turner et al (1968) reported the incidence of the HBsAg positivity in the nurses and technicians who were heavily jaundiced and the hepatitis associated antigen was also seen in the person who handles the haemodialysis Unit without having jaundice sera strongly positive for HBsAg contain as many as 10^{12} to 10^{13} particles per mo. Radioimmunoassay (RIA) is the best technique to demonstrate HBsAg (Hoofnagle, 1979). HBsAg appears in the serum usually 4 to 6 weeks after the initials infections, the range is 12 days to 24 weeks (Shiff, 1975 and Holland et al, 1975). The level of HBsAg peaks and falls before the the patient is clinically ill. Therefore, it is important

to test for Australia antigen as soon as the diagnosis of hepatitis is clinically suspected (Holland et al,1975). In general the antigen is present at the lower concentration during the acute phase and disappears during convalescence in an uncomplicated case. Anti HBsAg detected in children exposed to HBsAg with subsequent and more pronounced Rise. The anti HBsAg titre suggest an anamnestic response. People with an anamnestic reaction don't develop biochemical and clinical evidence of acute hepatitis. These anti bodies persist for at least one year or may be life long. HBsAb appears 2 weeks to 2 months after the disappearance of HBsAg (Hoofnagle, 1979).

Pal et al (1973) observed that the incidence of Australia antigen is significantly high in cases of fulminant hepatitis and all the positive cases had fatal outcome and they had definite history of parentral infection. However, Redker (1975) reported that the incidence of acute fulminant hepatitis is only one or two percent after acute viral hepatitis infection and it is more common following HBV infection but may also follow HAV infection.

The concept that acute viral hepatitis might in some instances lead to chronic hepatitis and/or cirrhosis is old one and is derived largely from autopsy data from serial biopsy studies in which the sequence of changes have been observed (Howard and Watson, 1947). Ruggieri et al (1947), Ratnoffod and Patekay (1955), Graig et al,

(1955) and MacDonald and Mallong (1958). The frequency of Australia antigen in patients of chronic active hepatitis has been reported by several workers from different countries (Wright et al, 1969; Naccarato et al, 1969; Blumberg et al, 1970 and Prince et al, 1970). The incidence was 25 percent in United State in 1969 and 67 percent in 1970; 34% in Italy and 26% in Great Britain. These findings suggested that frequency of Australia antigen is more in these countries. But other reports from the Great Britain, Denmark, Australia and Chilli suggested that Australia antigen is present in lower proportion of chronic active hepatitis in these countries (Prince et al, 1970) and Wright et al, 1970). However, it has not been determined whether these descripancies represent variations in immunological technique or a true geographic variation in frequency of SH antigen in these conditions.

Sherlock et al (1970) and Nelson et al(1971) found that serial biopsy specimen of the patients who undergo from the acute viral hepatitis to cirrhosis of the liver were having persistent antigenemia. Australia antigenaemia has been shown to be associated with 25 to 30 percent of patients with chronic hepatitis. Progression from hepatitis to cirrhosis with hepatoma has been recorded in an Australia antigen positive case (Pal et al, 1973).

It has been recognised that the large percentage of patients with chronic active hepatitis who have negative LE cell prepration who lack smooth muscles antibodies and have circulating HBsAg in their sera(Gitnick et al, 1969; Wright et al, 1963). About 1-3% of patients with viral hepatitis which are ill enough for hospitalization, will developed chronic hepatitis, which may be lead to cirrhosis. However, Kedeker (1975) has suggested that if initial hepatitis is fulminated patient rarely developed chronic hepatitis. Moslay (1975) reported that in chronic active hepatitis the HBsAg titre are usually low with marked fluctuations in contrast to the asymptomatic carrier where HBsAg titre are often very high. Clearance of HBsAg in a very good prognostic sign regardless of histology while Nelson (1971) reported that in 253 patients who are admitted in the copennagen Hospital, 113 were australian antigen positive for one to 13 weeks average 4.5 weeks. Australia antigen persisted for more than 13 weeks. In 11 of the 253 patients 8(4.3%) of them developed clinical and biochemical signs of chronic hepatitis and they were diagnosed as chronic aggressive hepatitis and 2 of them has chronic persistant hepatitis histologically.

The incidence of Australia antigen in cases of chronic persisting hepatitis as reported by different workers from different countries (Fox et al, 1969). Guardia (1970), Krassnitsky (1970) and Vischer (1970) have been markedly variable ranging from 0.14% to 5.3% by agar gel diffusion method (Alfred and Prince, 1971).

Chronic active hepatitis B often progress to cirrhosis an epidemiological associations between a high incidence HBsAg antigenemia and macronodular cirrhosis in certain tropical countries has been reported by William (1975). Significantly greater frequency of Australia antigen, than present in control proportion has been observed by most of the authors in cases of cryptogenic cirrhosis and post necrotic cirrhosis in different countries (Okochi and Murakami, 1968; Fox et al, 1969; Golke et al, 1969; Fobar et al, 1970; Prince et al, 1970 and Reinick and Nordenfelt, 1970).

In India Kelkar et al (1977) reported 34% positivity of Australia antigen in patients of cirrhosis by IEOP method. Pal et al (1973) reported 28% positiveness of australia antigen in post necrotic cirrhosis while other workers have reported that the incidence of Australia antigen in liver cirrhosis was low at Bombay (Baxi, 1972). Wright et al (1969) have observed 4% positiveness of Australia antigen in post necrotic cirrhosis.

The frequency of Australia SH antigen in cases of alcoholic cirrhosis to be significantly lower suggesting that this may be an etiologically lower distinct entity (Alfred and Prince, 1971). In primary biliary cirrhosis an antigen which appeared to be similar to or identical with SH was found in 9 out of 10 cases studied by Kroh et al (1970) and in one out of three cases reported by Naccarato et al (1969); while others could not detect Australia antigen in such cases (Fox et al,

1969; Wright et al, 1969; Reinick and Morden et al, 1970 and Vischar, 1970).

The frequency of Australia antigen in India childhood cirrhosis has been studied by Chandra (1970) who reported Australia antigen positivity in 20% cases.

More than 60% of all primary carcinomas of the liver occur in patients with pre-existing cirrhosis and more than 40% of autopsied cases of cirrhosis in Africa have co-existing carcinoma. Studies on the prevalence of Australia antigen carrier, state in cases of primary liver cancer are thus of special interest (Alfred and Prince, 1971).

The etiology of liver cell carcinoma is not known but recent research on the role of alpha toxin and hepatitis B virus strongly suggested that they may be involved, it is well known that liver cell carcinoma arises more often in cirrhotic liver. The frequency of liver cell carcinoma in various part of the world may differ by as much as 100 folds. In Europe and the United States the frequency varies from 0.1 to 0.7% of all autopsies. In portions of south east Asia, where the incidence of carcinoma is low usually between 4 to 6% rarely 10% of patients with cirrhosis eventually develop carcinoma of liver. This contrasts with some areas of Africa where the frequency is about 40% of all men with cirrhosis (Anderson, 1977). A higher familial incidence

of HBsAg positive liver cell carcinoma has been reported from Japan where the family members without carcinoma had an unusually high incidence of PVHB, CAVHB and active viral hepatitis B (Ohbayashi, 1976).

Various workers (Fox et al, 1969; Smith and Blumberg, 1969; Hadziyahnis et al, 1970; Prince et al, 1970; Vogal et al, 1970) from all over the world reported the frequency of 0 to 40% of Australia antigen in primary carcinoma liver. The maximum frequency was reported in Sangal 42% by (Prince et al, 1970 and Vogal et al, 1970) . From all over the world reported the frequency of 40% by Vogal et al, 1970 and 31% in France, while minimum frequency of Australia antigen by Prince et al, 1970 in USA (2 to 4% Hong Kong 5%, East Africa 0% and UK 5%) (Fox et al, 1969).

Anderson (1977) also reported that in Losangles the incidence of hepatocellular carcinoma increased over the past 30 years both in the cirrhotic and non cirrhotic liver. The percentage of cases in which HBsAg can be demonstrated in liver tissue has also increased and is currently found in sera of 20% of non alcoholic cirrhosis. Patients who developed cirrhosis and 30% with all patients with hepatocellular carcinoma. According to Smith and Blumberg (1969) the incidence of HBsAg was high in the hepatocellular carcinoma. There is high incidence of hepatocellular carcinoma in areas of world,

where the prevalence rate of HBs antigenemia is high. In Taiwan 90% hepatocellular carcinoma of HBsAg positive (William, 1975). Younger patients with hepatocellular carcinoma show HBsAg in malignant cells. Kaw et al (1979) reported that HBsAg and HBcab was reported in 61.6% and 90% of patients with HCC respectively. Szmuners et al (1975) have reported that approximately 1 out of 500 of HBsAg carrier developed HCC (Hepatocellular carcinoma).

MATERIAL AND METHODS

The present study was conducted on blood donors (voluntary and professional), all the cases of acute viral hepatitis and other liver diseases admitted in indoor medical wards of M.L.B. Medical College, Hospital, Jhansi, during the period from November, 1988 to September, 1989. All the cases were followed up till discharge/death during the period of study for any further complications. A detailed clinical history of each patient was recorded. Patients were especially asked about past history of any attack of jaundice, blood transfusion, intramuscular and intravenous injections, immunization, gamma globulin injection, surgical operations and anaesthetic administration. A detailed history of drug intake, like methyldopa, methotrexate, oxyphenacetin, oral contraceptives, anaesthetic agents, para aminosalicylic acid, Rifampicin and other hepatotoxic agents was noted.

A special note was taken of the dietary history of patient to assess the daily protein intake. Regarding personal history, alcohol intake, its frequency, amount and duration were noted in detail. Diseases like prolonged cholestasis, storage diseases, cardiac failure, haemochromatosis, syphilis and Wilson's disease were exlucded from this study. Cases of acute viral hepatitis and blood donors were subjected to a questionnaire and the observations were recorded in a working proforma (Annexures 1 and 2 respectively). A detailed clinical examination was done and a note was taken if any evidence of bleeding tendency, level

HBSAg negative HBSAg positive

of consciousness, severity of jaundice, liver size and any evidence of chronic liver disease, like palmer erythma, spider navi or signs of portal hypertension. Relevant laboratory investigations were performed in all the cases which included blood examination, for TLC, DLC, ESR, haemoglobin, blood urea and blood sugar. Bleeding time was determined by finger prick technique while coagulation time was done by Dane and Laidlow technique, Prothrombin time was done by Quick's one stage technique. Urine examination was done for presence of bile salts by Hay's test, while bile pigments and urobilinogen by Fouchet's test and Wallace and Diamond qualitative tests respectively or urine albumin, sugar and microscopic examination were also done.

Total serum bilirubin estimation was modification of Malloy Eveline method and thymol turbidity test(Macisgan, 1944). The conjugated and unconjugated bilirubin (these included Vandenberg test) were performed by the method of Malloy and Eveline (1937). Total serum protein, globulin and albumin were estimated by direct method (Kein Fiold, 1950).

All the three enzymes (Serum Asparate transminase, serum Alamine transaminase and serum alkaline phosphatase) were estimated by colorimetric method (Rietman and Frankel, 1957). In other cases of liver diseases, liver biopsy was done for confirmation of diagnosis.

TEST FOR HBsAg

Any test for HBsAg must be fulfill the following criteria :

1. The test must be rapid, since, blood has a definite storage life, result of HBsAg testing must be obtained quickly if the most efficient use is to be made of the blood.
2. The test must be specific, false positive results owing to interference from other antigen antibody reactions will result in blood being rejected. A large proportion of false positive would obviously cause considerable wastage of blood.
3. The test must be sensitive and capable of detecting minute amount of antigen. Generally as sensitivity increases, specificity decreases and a test system must be used which achieves a balance between these two.

DETECTION OF HBsAg

Various methods are in use for the detection of HBsAg. They include :

1. Immunoelectro-osmophoresis(IEOP) (WHO, 1979).
2. Micro ouchterlony Agar gel immunodiffusion (I.O.) (WHO, 1970).
3. Single Radial Immunodiffusion(RID)(Mancini *et al*, 1965).
4. C.I.E.P. (Counterimmunoelectrophoresis).
5. Radio immuno assay.
6. R.P.H.A. Test : (Reverse Passive Haem-agglutination Test).
7. ELISA Test (Enzyme linked immunosorbent assay) Walter *et al*, 1976; Wei *et al*, 1977; Abelvert

and Anken, 1977).

8. Latex Agglutination test.

LATEXT AGGLUTINATION TEST

Principle

Latex particles are coated with gamma globulin from rabbit containing highly purified antibodies with high reactivity for HBsAg. By mixing serum containing HBsAg with Latex reagent a distinct agglutination will occur. There is no agglutination if HBsAg is not present this test is a 3rd generation test according to specifications of FDA of U.S.A. (Sensitivity for subtypes ad - 10 ug/ml, sensitivity for subtype ay - 20 ug/ml).

Sample

It is recommended that the test be performed on serum. Don't heat inactivate the serum. Store test sera in a refrigerator or a deep freezer, if delay is anticipated before testing. Avoid repeated freezing and thawing of the specimen.

Reagents and Accessories needed :

Reagents :
1. HBsAg latex reagent.
2. Positive control serum.
3. Negative control serum.

Accessories :
1. Disposable plastic slides.
2. Disposable applicators sticks.
3. Disposable plastic droppers.
4. Rubber teats.

STORAGE AND STABILITY

All reagents are stable at 2-8°C till expiry date mentioned on the individual label. Don't freeze.

USE OF CONTROLS

Positive and negative controls are not always required when the reagents are in continuous use or the variety of specimen being tested will ensure both agglutinated and unagglutinated patterns frequently. However, such controls are provided in the kit for performing an occasional check. It is therefore, not necessary to run positive and negative controls with every test. Don't dilute controls.

PROCEDURE

First we bring reagents at room temperature and mix gently before use. Make sure that Latex reagent is completely in suspension and then we proceed as follows:

1. Place one drop (50 ul) of undiluted serum within appropriate circle on the slide.
2. Mix Latex reagent gently for complete resuspension of particles and add one drop to the above drop of serum on the slide.
3. Mix with the disposable applicator stick and spread uniformly over the entire area of the circle.
4. Tilt the slide back and forth slowly for 5 minutes and watch for agglutination.

INTERPRETATION :

1. HBsAg negative - No agglutination.

2. HBsAg positive: visible agglutination within 5 minutes.

ADVANTAGES

1. Test is simple, rapid and specific.
2. It is ideal, screening test for manual procedure.
3. Results available after 5 minutes.
4. Sensitivity of 3rd generation test is good.
5. No pre-treatment of sample is necessary.
6. Latex reagent is stable for 1 year.
7. Less than 1% false positive.
8. End point is distinct and easy to read.

PRECAUTIONS

1. Allow all the reagents to attain room temperature before use.
2. It is necessary to exercise great care to avoid contamination of reagents make sure that the cap of each vial is properly and promptly applied to the same vial. Interchanging of caps and/or droppers will lead to contamination of reagent which might lead to false results.
3. Improper mixing of sample with the reagents will lead to erroneus results.
4. The slide should be rocked gently since vigorous rocking may impair agglutination.
5. Drying of the reagents on the slide may lead to erroneus results.

OBSERVATIONS

The present study was conducted at Maharani Laxmi Bai Medical College, Hospital, Jhansi(UP). The study comprised of 50 cases of liver diseases, viz. acute viral hepatitis (AVH), chronic active hepatitis (CAH), chronic persistent hepatitis, cirrhosis and hepatoma, and 60 professional and 100 voluntary blood donors.

All 50 cases of liver diseases were admitted in M.L.B. Medical College, Hospital, Jhansi during a period of 11 months from Nov., 1988 to Sept., 1989.

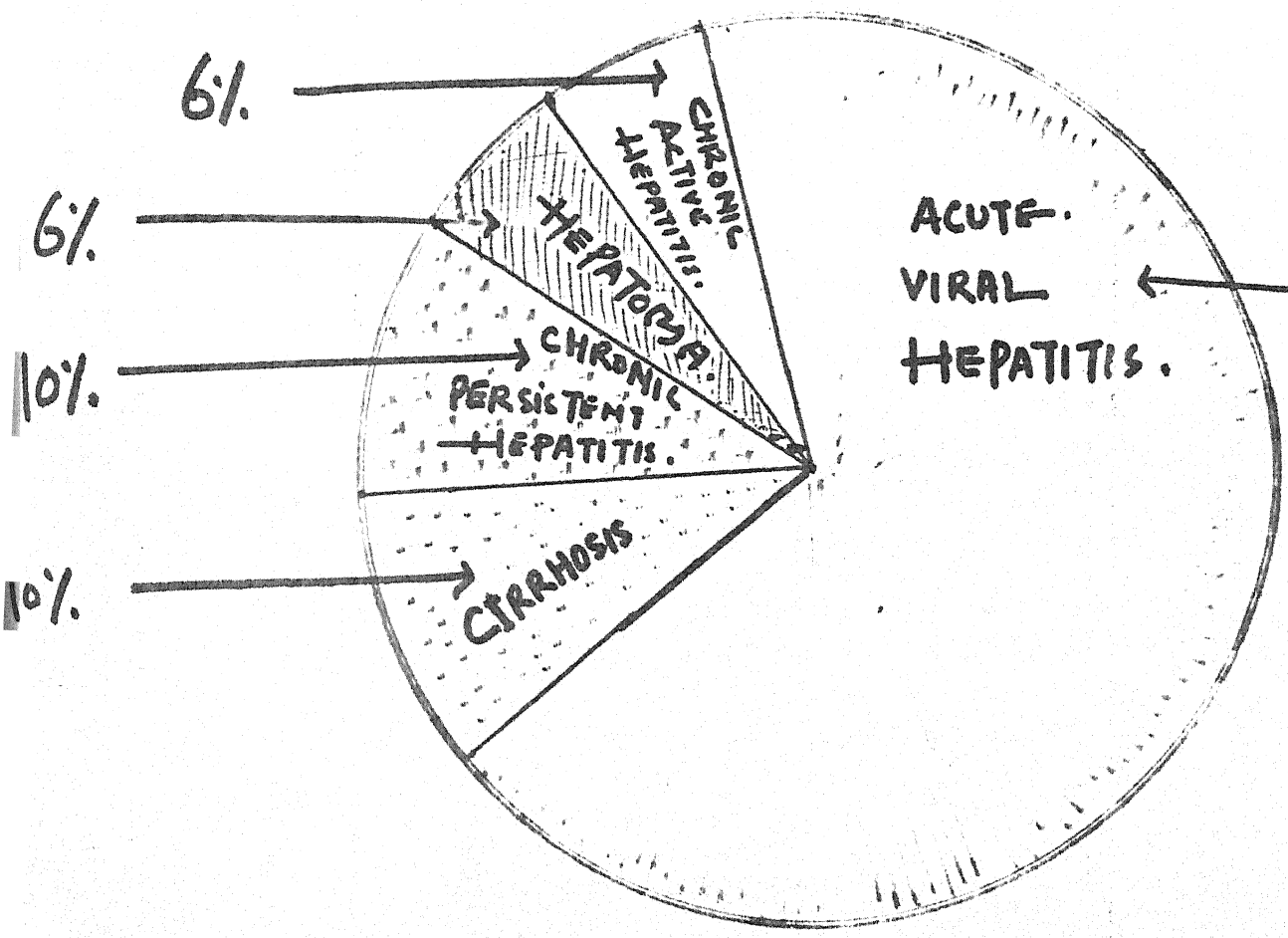
The different groups of liver diseases which were studied are depicted in table 1.

TABLE 1 : Showing the number of cases in different groups of liver disease.

Groups	Male	Female	Total
Acute viral hepatitis	21	13	34
Chronic active hepatitis	1	2	3
Chronic persistent hepatitis	3	2	5
Cirrhosis	3	2	5
Hepatoma	2	1	3
Total	30	20	50

The maximum number 34(68%) of cases belonged acute viral hepatitis while the least number of cases 3 (6%) were of hepatoma. Out of 34 cases of acute

PIE DIAGRAM SHOWING DISTRIBUTION OF DIFFERENT LIVER DISEASES



ACUTE VIRAL HEPATITIS.

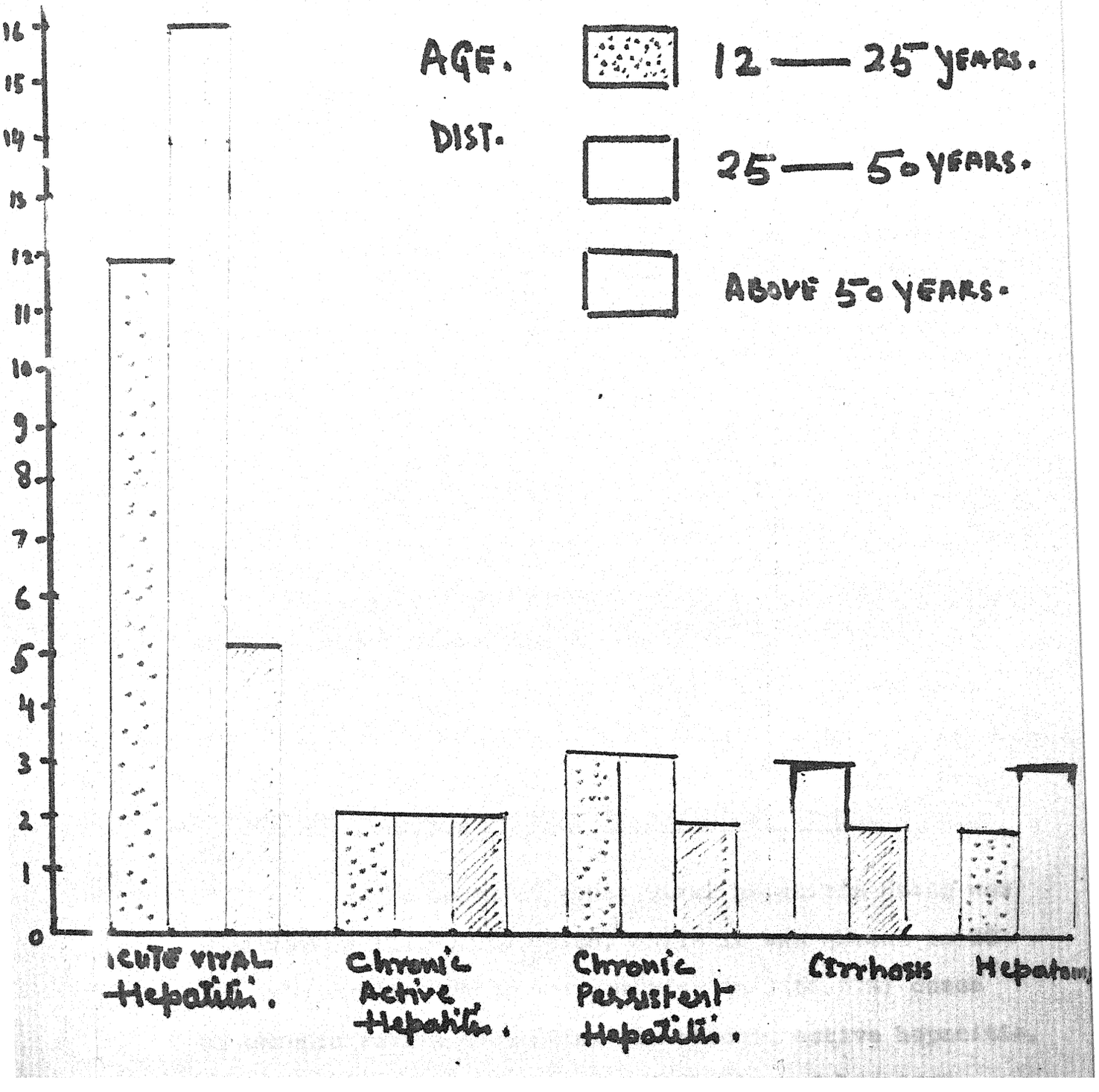
CIRRHOSIS.

CHRONIC PERSISTENT HEPATITIS.

HEPATOMA.

CHRONIC ACTIVE HEPATITIS.

DIAGRAM SHOWING AGE DISTRIBUTION OF DIFFERENT LIVER DISEASES.



viral hepatitis, 21 (63%) were males and rest 13(37%) were females. Out of the total of 3 cases diagnosed as chronic active hepatitis, 1(33.33%) was a male and the remaining 2 cases (66.67%) were females. In chronic persistent hepatitis group there were 3(60%) males and 2(40%) females.

TABLE 2 : Showing the age distribution of different disease groups.

Age in years	Acute viral hepatitis	Chronic active hepatitis	Chronic persistent hepatitis	Cirrhosis	Hepatoma	Total
12 - 25*	12	1	2	-	-	15
25 - 50	16	1	2	3	1	23
7 50	6	1	1	2	2	12
Total	34	3	5	5	3	50

* Cases below 12 years (Paediatrics group) were not included in the study.

The maximum number of cases (23, 46%) belong to age group 25-50 years. Cirrhosis and hepatoma cases were usually of higher age group than others.

HEPATITIS B SURFACE ANTIGEN IN DIFFERENT DISEASE GROUPS

In 34 cases of acute viral hepatitis HBsAg was positive in 12(34.12%) cases, while it was absent in 22 (65.88%) cases. HbsAg was positive in 2(66.67%) cases of chronic active hepatitis. In chronic active hepatitis, chronic persistent hepatitis, cirrhosis and hepatoma

2(66.67%), 2(40%) and 1(33.33%) patients respectively were HBsAg positive (Table 3).

TABLE 3 : Showing hepatitis B surface antigen positivity in different groups.

Groups	Total No.of cases	HBsAg positive cases	
		No.	(%)
Acute viral hepatitis	34	12	34.12
Chronic active hepatitis	3	2	66.67
Chronic persistent hepatitis	5	2	40.00
Cirrhosis	5	3	60.00
Hepatoma	3	1	33.33
Total	50	20	40.00

From the table 3, it is evident that maximum positivity of HBsAg was present in cases of chronic active hepatitis (66.67%), cirrhosis (60%) and chronic persistent hepatitis (40%). HBsAg was also present in 34.12% cases of acute viral hepatitis. The over all positivity of HBsAg was 40%.

HBsAg IN RELATION TO CLINICAL PROFILE OF DIFFERENT DISEASE GROUPS

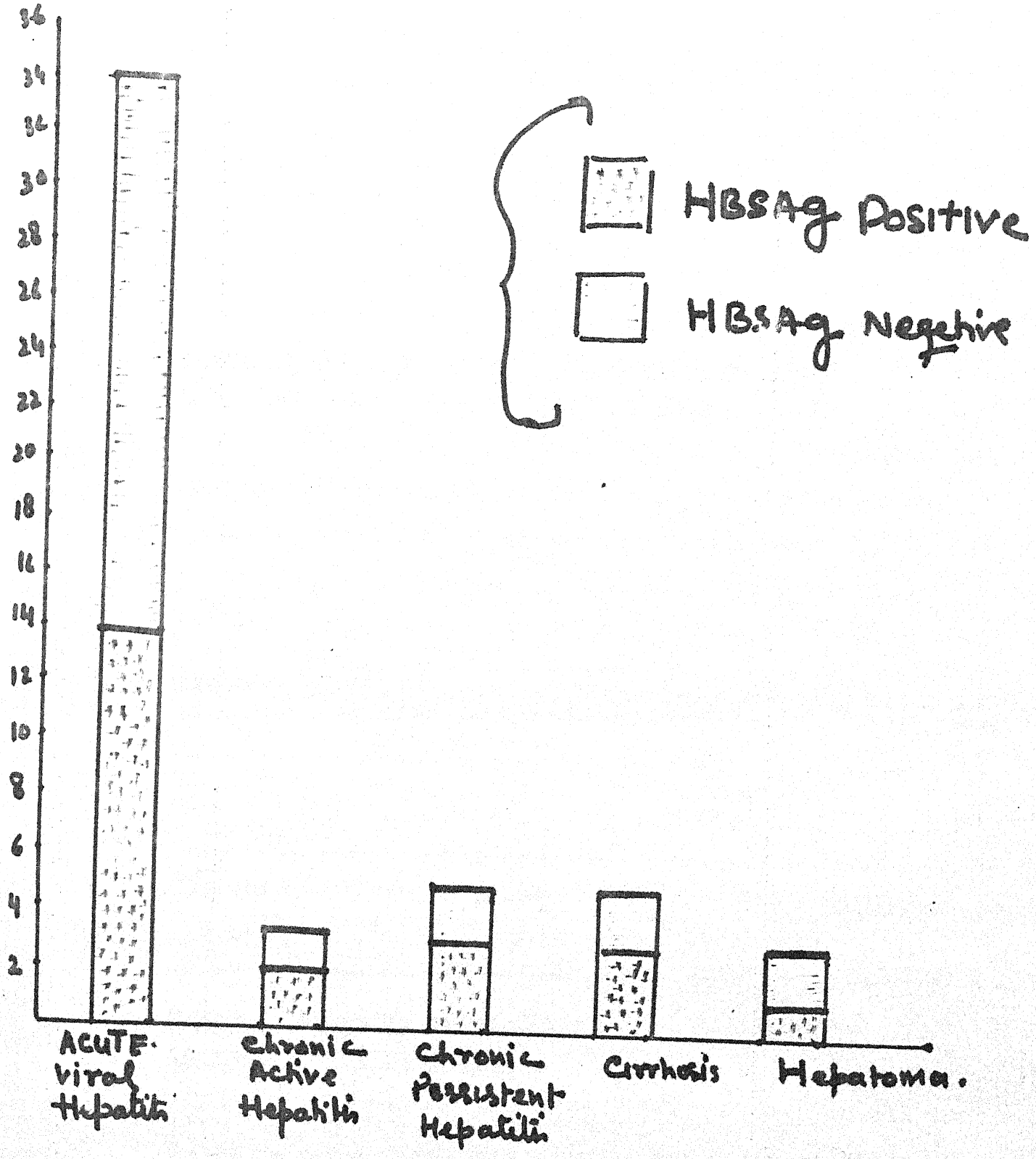
A. ACUTE VIRAL HEPATITIS

Table 4 shows the HBsAg in relation to previous history of jaundice in acute viral hepatitis.

Out of 12 HBsAg positive cases as many as 8(66.67%) cases had past history of jaundice. In HBsAg negative cases, on the other hand history of jaundice

COMPANENT BAR DIAGRAM SHOWING HBSAg.

POSITIVITY IN DIFFERENT LIVER DISEASES.



was present in only 2(9.1%) cases. This difference was highly significant (X^2=12.5, d.f.=1, p $<$0.001).

TABLE 4 : Showing HBsAg in relation to past history of jaundice in cases of acute viral hepatitis.

Past history of jaundice	HBsAg positive cases		HBsAg negative cases		p value
	No.	(%)	No.	(%)	
Present	8	66.67	2	9.10	$<$0.001
Absent	4	33.33	20	90.90	

HBsAg in Relation of Previous History of Blood Transfusion in cases of Acute Viral Hepatitis

Out of HBsAg positive cases 4(33.33%) cases had past history of blood transfusion. In HBsAg negative cases history of blood transfusion was present in 2(9.1%) cases. This difference was statistically significant (X^2 = 6.92, d.f. = 1, p $<$0.01).

TABLE 5 : Showing the relationship of HBsAg and previous history of blood transfusion in cases of Acute viral hepatitis.

History of blood transfusion	HBsAg positive cases		HBsAg negative cases		p value
	No.	(%)	No.	(%)	
Present	4	33.33	2	9.10	$<$0.01
Absent	8	66.67	20	90.90	

HBsAg in Relation to Symptoms in Acute Viral Hepatitis

The history of jaundice was present in all the cases of acute viral hepatitis irrespective of HBsAg.

Anaemia was noted in 8(66.67%) cases of HBsAg positive group and it was present in 14(64%) cases of HBsAg negative group. These values were statistically insignificant (X^2=0.012, d.f. = 1, p >0.05). Out of 12 HBsAg positive cases 3(25%) had pedal oedema and it was present in 4(18%) cases of HBsAg negative group. All these values were statistically insignificant (X^2 = 0.195, d.f. = 1, p >0.05).

A history of gastro-intestinal haemorrhage, i.e. haematemesis or malena was present slightly in higher percentage in HBsAg positive group i.e. 4(33.33%) cases of the HBsAg positive and 6(26%) of HBsAg negative group. These values were statistically insignificant (X^2 = 1.144, d.f. = 1, p >0.05).

TABLE 6 : Showing HBsAg in relation to various symptoms in acute viral hepatitis(N=34).

Symptoms	HBsAg positive cases(n=12)		HBsAg negative cases(n=22)	
	No.	(%)	No.	(%)
Jaundice	12	100.00	22	100.00
Anaemia	8	66.67	14	64.00
Oedema	3	25.00	4	18.00
Haematemesis	4	33.33	6	26.00
Ascites	4	33.33	6	26.00

Out of 12 cases of HBsAg positive group, 4(33.33%) cases had mild ascites and on the other hand it was present in 6(26%) cases of HBsAg negative group.

This finding is statistically insignificant (X^2=0.144, d.f. = 1, p 70.05). On the basis of symptoms, we cannot predict the HBsAg positivity or negativity.

HBsAg in relation to various Physical Sings of Acute Viral Hepatitis

The relationship of various signs of acute viral hepatitis cases with HBsAg positivity is depicted in table 7.

The jaundice was nearly equally prevalent in both groups, i.e. present in 10(83.34%) of the HBsAg positive and in all the 22 (100%) cases of HBsAg negative acute viral hepatitis cases.

TABLE 7 : HBsAg in relation to various signs of acute viral hepatitis (N=34).

Signs	HBsAg positive cases (n=12)		HBsAg negative cases (n=22)	
	No.	(%)	No.	(%)
Icterus	10	82.34	22	100.00
Pallor/Anaemia	7	58.33	15	68.20
Hepatomegaly	8	66.67	16	72.72
Splenomegaly	2	16.66	3	13.60

Anaemia was noted in 7(58.33%) cases of HBsAg positive group and in 15(68.2%) cases of HBsAg negative group. The values were statistically insignificant (X^2=0.344, d.f. = 1, p 70.05).

Splenomegaly, an evidence of portal hypertension was noted in 2(16.66%) of the HBsAg positive group and in 3(13.6%) cases of HBsAg negative group. These findings were in accordance that HBsAg positivity has not bearing

regarding the presence or absence of splenomegaly. The values were statistically insignificant (X^2=0.128, d.f.=1, p >0.05). Hepatomegaly was seen in 8(66.67%) cases of HBsAg positive and 16(72.72%) in HBsAg negative group. The results were statistically insignificant (X^2 = 0.150, d.f. = 1, p >0.05).

Our observations indicate that clinical features of HBsAg positive and HBsAg negative acute viral hepatitis were quite similar except a previous history of jaundice which suggest HBsAg positive hepatitis.

B. CHRONIC ACTIVE HEPATITIS, CHRONIC PERSISTENT HEPATITIS CIRRHOSIS AND HEPATOMA

TABLE 8 : Showing relationship of various symptoms of chronic hepatitis, cirrhosis and hepatoma and positivity of HBsAg(N = 16)

Symptoms	HBsAg positive cases(n=8)		HBsAg negative cases(n=8)	
	No.	(%)	No.	(%)
Jaundice	6	75.00	4	50.00
Peritoneal tapping	4	50.00	3	37.50
Swelling over feet	5	62.50	2	25.00
Loss of livido	2	25.00	3	37.50
Haematemesis or malena	6	75.00	4	50.00

In the HBsAg positive group of chronic hepatitis cirrhosis and hepatoma, 6(75%) cases gave a history of jaundice. On the other hand in the HBsAg negative group 4(50%) cases gave such history. The above values indicate apparently that a history of jaundice may

increase the probability of its positivity. regarding HBsAg but the values were statistically insignificant (X^2 = 1.06, d.f. = 1, p 70.05).

Out of that 8 of HBsAg positive chronic hepatitis, cirrhosis and hepatoma cases 4(50%) cases gave a past history of peritoneal tapping. The same values in HBsAg negative group were 3(37.50%). The values were statistically insignificant (X^2=0.242, d.f. = 1, p 70.05). Similar values were noted in case of a history of pedal oedema hence it also does not have any influence regarding positivity in cases of chronic hepatitis, cirrhosis and hepatoma (X^2 = 2.284, d.f. = 1, p 70.05). A history of the HBsAg positive group and it was normal in 6(75%) cases, while the values for HBsAg negative group were 3(37.5%) and 5(62.5%) respectively. Values were statistically insignificant (X^2 = 0.290, d.f. = 1, p 70.05).

A history of haematemesis or malena was seen in 6(75%) cases of the 8 positive group and 4(50%) in HBsAg negative group. The values were statistically insignificant (X^2 = 1.06, d.f. = 1, p 70.05).

On the basis of symptoms we cannot predict the HBsAg positivity or negativity in chronic active hepatitis chronic persistent hepatitis, cirrhosis and hepatoma.

Table 9 shows the relationship of various signs of chronic hepatitis, cirrhosis and hepatoma and HBsAg positivity.

Jaundice was present in 7(87.5%) cases of chronic hepatitis, cirrhosis and hepatoma who showed HBsAg positivity and 3(37.5%) in cases of HBsAg negative group. These values are statistically significant (X^2=4.26, d.f.=1, p $<$0.05) which indicate that presence of jaundice in a case of chronic hepatitis, cirrhosis and hepatoma has definitely bearing regarding the positivity of HBsAg.

TABLE 9 : HBsAg in relation to various signs of chronic hepatitis, cirrhosis and hepatoma (N = 16).

Signs	HBsAg positive cases (n=8)		HBsAg Negative cases (n=8)	
	No.	(%)	No.	(%)
Jaundice	7	87.50	3	37.50
Anaemia (Pallor)	6	75.00	6	75.00
Collateral circulation	3	37.50	2	25.00
Ascites	4	50.00	3	37.50
Splenomegaly	4	50.00	3	37.50
Hepatomegaly	2	25.00	2	25.00

Out of the 8 cases of HBsAg positive group anaemia was present in 6(75%). The similar values for HBsAg negative group were 6(75%). The values were statistically insignificant (X^2=00, d.f.=1, p $>$0.05). Signs of collateral circulation were noted in 3(37.5%) cases of HBsAg positive group and 2(25%) in cases of HBsAg negative group. This indicate that there is no correlation regarding the collateral circulation

and HBsAg positivity. The results were statistically insignificant (X^2=0.253, d.f.=1, p >0.05).

Splenomegaly, a sign of portal hypertension was present in 4(50%) cases of the 8 cases who showed the presence of HBsAg. The values for HBsAg negative group were 3(37.5%) and the values were not statistically significant (X^2=0.253, d.f.=1, p >0.05) which indicate that presence or absence of splenomegaly had no bearing regarding the HBsAg positivity in cases of chronic hepatitis, cirrhosis and hepatoma.

Hepatomegaly was present in 2(25%) cases of HBsAg positive group and 2(25%) in HBsAg negative group. The values were statistically insignificant (X^2=0, d.f. = 1, p >0.05).

RELATIONSHIP OF HBsAg AND HEPATIC ENCEPHALOPATHY IN LIVER DISEASES

TABLE 10 : Relationship of HBsAg positivity and hepatic encephalopathy in liver diseases (N=14).

Hepatic encephalopathy	HBsAg positive cases (n=20)		HBsAg negative cases (n=30)	
	No.	(%)	No.	(%)
Present	12	60.00	2	6.66
Absent	8	40.00	28	93.34

Out of 20 cases of positive HBsAg, 12 (60%) showed signs of hepatic encephalopathy. On the other hand the values for negative group were 2(6.66%)

The values were statistically highly significant (X^2= 16.91, d.f. = 1, p $<$0.001) which indicate that hepatic encephalopathy has definite bearing regarding the positivity of HBsAg.

HBsAg IN RELATION TO VARIOUS LIVER FUNCTION TESTS IN DIFFERENT DISEASE GROUPS

A. Acute Viral Hepatitis

Table 11 shows biochemical profile in relation to HBsAg in acute viral hepatitis cases. Out of 12, HBsAg positive cases of acute viral hepatitis the serum bilirubin level ranged between 1.5 - 20 mg% with a mean of 11.2±3.6 mg% while in the HBsAg negative group the range was 4 - 18 mg% and mean 7.2±3.1 mg%. These values were statistically significant (t=3.41, d.f.=32, p $<$0.05) which indicate serum bilirubin levels have definitely a bearing regarding the HBsAg positivity in acute viral hepatitis.

The total serum protein levels ranged between 4.7 to 8.1 gm% with a mean of 7.6±0.8 gm% in HBsAg positive group, while the values in HBsAg negative group were 5.2-7.2 gm% and 6.2±0.6 gm% respectively. These values were statistically not significant (t = 0, d.f. = 32, p $<$0.05).

Serum albumin levels were noted ranging between 2.4 to 4.5 gm% with a mean of 3.6±0.7 gm% and 3 to 7.5 gm% with a mean of 3.86±0.5 gm% in cases of HBsAg

positive and HBsAg negative group respectively. These values are statistically not significant (t=0.981, d.f.= 32, p $\nearrow$0.05). Out of 12 HBsAg positive cases of acute viral hepatitis a mean serum globulin value come to be 2.8 to 3.6 gm% with 3.1$\pm$0.5 gm% and in negative group 2 to 3.5 gm% and 2.9$\pm$0.05 gm% respectively. These values are statistically insignificant (t=1.117, d.f.=32, p $\nearrow$0.05). The level of serum glutamine oxaloacetic acid transaminase (SGOT) ranged between 11-60 IU with a mean of 30.2$\pm$18.4 IU in HBsAg positive group while the values in the negative group were 12-56 and 14.3$\pm$6.9 IU respectively. The values are statistically significant (t = 2.396, d.f. = 32, p $\swarrow$0.05).

The level of SGPT ranged between 12-64 IU and with a mean of 28.4$\pm$16.4 IU in HBsAg positive group while in negative group they were 13 to 66 and 19.8 $\pm$5.6 IU respectively. The values are just statistically significant (t=2.04, d.f. = 32, p $\swarrow$0.05).

The data are statistically insignificant in relation of serum alkaline phosphatase (= t = 1.02, d.f. = 32, p $\nearrow$0.05).

TABLE 11 : Acute viral hepatitis (Biochemical profile) Liver function tests (N=34).

Liver function tests	HBsAg positive cases (12)	HBsAg negative cases (22)
Serum bilirubin (mg%)		
Range	1.5-20	4-18
Mean±SD	11.2±3.6	7.2±3.1
Total serum proteins (gm%)		
Range	4.7-8.1	5.2-7.5
Mean±SD	7.6±0.8	6.2±0.6
Serum albumin (gm%)		
range	2.4-4.5	3.0-4.5
Mean±SD	3.6±0.7	3.8±0.5
Serum globulin (gm%)		
Range	2.8-3.6	2.0-3.5
Mean±S.D.	3.1±0.5	2.9±0.5
S.G.O.T. (IU)		
Range	11-60	12-56
Mean±SD	30.2±18.4	14.3±6.9
S.G.P.T. (IU)		
Range	12-64	13-66
Mean±SD	28.4±16.4	19.8±5.6
S. Alkaline Phosphatase (KAU)		
Range	6-20	3.3-20.0
Mean±SD	7.3±3.2	6.3±2.5

B. CHRONIC HEPATITIS, CIRRHOSIS AND HEPATOMA

Table 12 shows the biochemical profile in relation of HBsAg in chronic hepatitis, cirrhosis and hepatoma cases.

TABLE 12 : Biochemical profile of chronic hepatitis cirrhosis and hepatoma in relation of HBsAg (N=16).

Liver function tests	HBsAg positive cases	HBsAg negative cases
Serum bilirubin (mg%)		
Range	1.5-17.0	2.2-15.0
Mean±SD	6.8±3.8	6.7±3.2
Total serum protein (gm%)		
Range	5.5-7.3	6.1-7.4
Mean±SD	6.2±0.8	6.3±0.7
Serum Albumin (gm%)		
Range	2.2-3.6	2.8-4.2
Mean±SD	2.9±0.4	3.1±0.6
Serum Globulin (gm%)		
Range	2.4-4.2	2.2-4.4
Mean±SD	3.3±0.5	3.2±0.5
S.G.P.T. (IU)		
Range	9-32	6-24
Mean±SD	16±4.9	11±2.3
S.G.O.T. (IU)		
Range	8-30	9-28
Mean±SD	14±6	10.7±3
S. Alkaline Phosphatase (KAU)		
Range	3-16	3.3-18.0
Mean±SD	7.1±2.5	5.9±3.0

The values of serum bilirubin in the HBsAg positive group ranged between 1.5-17 with mean of 6.8±3.8 mg% and the corresponding values in HBsAg negative group were 2.2-15 and 6.7±3.2 mg% respectively.

Values are statistically insignificant (t=0.056, d.f. = 14, p >0.05). t values are also evaluated for total serum protein, albumin and globulin. They are t = 0.191, d.f. = 14, >0.05; t=0.067, d.f.=14, p >0.05 and t = 0.8, d.f. = 14, p >0.05 respectively.

Serum glutamine oxalacetic acid transaminase (SGOT) were ranging between 8-30 IU with mean of 14±6 IU and for HBsAg negative group values were 9-28 IU with mean of 10.7±3 IU respectively. Values are statistically significant (t=2.78, d.f. = 14, p <0.05). The values of serum glutamin pyrivic transaminsae (SGPT) were ranged 9-32 IU with a mean of 16±4.9 IU while for negative group range was 6-24 IU with mean of 11±2.3(IU). The values are statistically significant(t=5.23, d.f.= 14, p <0.05). The serum alkaline phosphatase levels were found with the range of 3-16 KAU with mean of 7.1±2.5 KAU in HBsAg positive group while HBsAg negative group 3.3-18 KAU and 5.9±3 KAU respectively. Value was statistically insignificant (t = 1.76, d.f.=14, p >0.05) which indicate that levels of serum alkaline phosphatase have no relation with the HBsAg positivity in cases of chronic active hepatitis, chronic persistent hepatitis cirrhosis and hepatoma.

HBsAg IN RELATION TO HOSPITAL STAY AND MORTALITY IN ACUTE VIRAL HEPATITIS

Table 13 compares the relationship of HBsAg to mean hospital stay and mortality in acute viral

hepatitis . It was found that the duration of mean hospital stay was significantly higher in HBsAg positive group ($t = 3.42$, $d.f.=32$, $p < 0.05$). None of the 22 HBsAg negative patient died. There were 2 (16.66%) deaths in HBsAg positive patients. However the difference was statistically insignificant ($P > 0.05$).

TABLE 13 : Relationship of HBsAg to hospital stay and mortality in acute virhal hepatitis.

	HBsAg positive cases (n=12)	HBsAg negative cases (n=22)
Hospital stay Mean±SD (days)	24±13.2	14±7.4
Mortality	2 (16.66%)	-

TABLE 14 : Showing Australia antigen positivity amongst the professional and voluntary blood donors.

Blood donors	Total No.	HBsAg positive No.	(%)
Professional	60	1	1.66
Voluntary	100	-	-

In the above table 14, voluntary blood donors who were medical student, ward boys, nurses and patient's attendants, all were negative for HBsAg whereas, the professional donors whose number was less, there was 1(1.66) case positive for HBsAg in professional donors. There is no statistical significance because number of the both groups areless.

All the professional blood donors were males and none of them had definite notion of their age, though most of them professed to be in the third and fourth decade of life. Analysis of age under the circumstances would prove meaningless.

Voluntary blood donors were predominantly males and all were above 16 and below 45 years of age.

TABLE 15 : Showing relationship of Australia antigen and ABO grouping amongst the professional and voluntary blood donors.

Blood group	No.of cases	Perce-ntage	HBsAg positive No.	(%)
Professional donors				
A	15	25.00	-	-
B	30	50.00	1	1.66
AB	5	8.33	-	-
O	10	16.67	-	-
Voluntary donors				
A	18	18.00	-	-
B	50	50.00	-	-
AB	6	6.00	-	-
O	26	26.00	-	-

In both the groups the individuals were predominantly of 'B' blood group. One case of HBsAg positive in professional blood donors also had 'B' blood group.

DISCUSSION

Mac Callum (1951) showed that there were at least two viral agents producing hepatitis having no cross immunity between them which may later on be converted into chronic liver disease. Later on Blumberg et al (1965) confirmed the above fact by discovering the Australia antigen which is associated only with large incubation serum hepatitis or virus B hepatitis.

The physical properties of the hepatitis B antigen were later on established. Its detection and demonstration was done by agar gel (Blumberg et al, 1965), complement fixation test (Parcell et al, 1959), Electron microscopy (Shulman and Backer, 1969). Immunofluorescence (Millman et al, 1970), Radio immune assay technique (Walsh et al, 1970), electro-osmodiffusion (Porsendorfer et al, 1970) and Immuno adherence test (Mayami et al, 1971).

In the present study we have investigated the patients of liver diseases and blood donors(professional and voluntary) for the presence of HBsAg using the latex agglutination test. A total of 210 individuals have been studied. Out of these 210 cases, there were 50 patients of liver diseases (acute viral hepatitis, chronic active hepatitis, chronic persistent hepatitis, cirrhosis and hepatoma) and 160 cases were blood donors (professional-60 and voluntary-100). The liver disease

group showed the overall prevalence of Australia antigen to be 40%. In the blood donor group prevalence of Australia antigen carriers among professional donors was 1.66%. None of the voluntary donor was positive for Australia antigen.

The exact relation of hepatitis antigen reported by Blumberg London et al (1969) and of the surface antigen of Prince (1968a) might expect to find several related but immunologically distinct antigens with different frequencies, disease associations and geographical distribution. Blumberg and his associates(1969) reported that 13% of patients with active hepatitis and 34% of patients with post transfusion hepatitis had Australia antigen (London et al, 1969). Then using heterologous antisera prepared in rabbits, they discovered an additional specificity associated with Australia antigen which was present in 70% of patients of acute viral hepatitis (Levene and Blumberg, 1969).

A report from Copenhagen hospitals showed that 4.3% of patients admitted as Acute viral hepatitis were HBsAg positive and all of them acquired chronic hepatitis (Nelsen et al, 1971). As stated by Sutnick et al,(1971) the susceptibility to persistent Australia antigen in serum is 3-4% in general population. It is also found that an Australia antigen carrier state in some patients may be due to a gene dependent susceptibility(Blumberg et al , 1969). A number of studies on the frequency of

HBsAg in hepatocytes in patients with cirrhosis of liver reveals a wide variation 0-49%(Seth and Nayak, 1975; Prince et al, 1970; Fox et al, 1969; Mathew et al, 1970; Van Waes et al, 1974, Kelkar and sharma, 1972 and Harsal 1983). Studies from USA (Prince et al, 1970); England (Fox, 1969) and Australia (Mathew et al, 1970) showed only 0-2% incidence in general population. Reports from several African countries (Kelkar and Sharma, 1972) as well as those from some European countries (Van Waes et al, 1974) and earlier studies in India (Kelkar, 1972) have frequencies ranging from 20-49 fairly correlating with our study.

TABLE 14 : Showing HBsAg prevalence rate in acute viral hepatitis.

Authors	Percentage of HBsAg positivity
1. Goeke et al (1969) New York	80.00
2. Nielson (1971) Denmark	44.00
3. Sehgal and Aiket (1970) India	54.00
4. Sama et al, (1971) India (Delhi)	12.00
5. Pal et al (1973) India(Chandigarh)	12.00
6. Hillis et al (1970) India	12.50
7. Mahadeva et al (1973) India (Delhi)	17.90
8. Ross and Mc Michael (1970) N.W.	12.60
9. Dutta and Mohammed (1972) India	20.68
10. Kelkar et al (1973) India (Poona)	16.50
11. Sama et al (1973) India	59.20
12. Present Study, Bundelkhand Region, India	34.12

In cases of acute viral hepatitis there has been a wide variation in the positivity of HBsAg in India (ranging between 12-59.2%)(Table 14) due to variation in prevalence rate of hepatitis B in different geographical areas. Sama et al (1973) reported a prevalence of 59.2% while Chaddha et al (1982) found it to be 16.7% only. We observed a prevalence of 34.12%. Our results are in good correlation with most of the other studies. Some western workers have reported figures as high as 80% (Goeke et al, 1969). The difference of prevalence rates in various studies are explained on the basis of the difference of criteria of selection of patients and the time of drawing the sample for the test.

In our study, in acute viral hepatitis, a past history of jaundice was statistically highly significant (X^2=12.5, d.f.=1, p $<$0.001). However, history of haematemesis, malena or immunization was found to have no bearing on the positivity of HBsAg. There was history of blood transfusion in 8(66.67%) of the 12 HBsAg positive cases. This value was also statistically significant (X^2=3.55, d.f.=1, p $<$0.05). On statistical analysis of the observation of biochemical profile of acute viral hepatitis which is mentioned in 11, it was inferred that levels of serum bilirubin were significantly higher in HBsAg positive than in HBsAg negative group (t=2.398, d.f.=32, p $<$0.05) and jaundice lasted

for longer duration in HBsAg positive patients. Serum aspartate aminotransferase and serum alamine aminotransferase were also statistically significantly higher in HBsAg positive group ($t=2.398$, d.f.=32, $p < 0.05$ and $t=2.04$, d.f.=32, $p < 0.05$ respectively).

No other biochemical test showed any significant variation in relation with HBsAg positivity. In chronic liver diseases (cirrhosis, chronic active hepatitis, chronic persistent hepatitis and hepatoma) study was done on the positivity of HBsAg in each subgroup separately and in combination. In present study a positivity was found to be 60% in cirrhosis subgroup. Seth et al (1975) have reported a 43% positivity while priyadarshini et al (1979) have reported 56.1%. The results of these workers nearly correlate with the observations of the present study. Chaddha et al (1982) and Aiket et al (1977) reported a lower prevalence of 22% and 23% positivity in their studies. This can be explained on the basis of different prevalence of hepatitis B infection in these geographical areas. In the chronic active hepatitis subgroup of the present study, 66.67% HBsAg positivity was noted. This is higher than those of Aiket et al (1977) who reported prevalence rates of 19% and 12.5% respectively. However, the small number of patients in our study (n=5) prevents any meaningful comparison.

In the hepatoma subgroup, the HBsAg positivity noted was 33.33%, which is higher in comparison to 21.7% reported by Priyadarshini et al (1979). Aiket et al (1977) have reported a positivity of 14% only while, it was 4.8% in a series of Chaddha et al (1982). The discrepancy of results in this study with others may be attributed to small sample size.

In chronic persistent hepatitis subgroup of the present study, the positivity for HBsAg was 40%. Deodhar et al (1975) reported positivity of 63.6% in their study. Carrella et al (1972) have reported a positivity of 16.6% by complement fixation and 11.1% by immunodiffusion technique. The results are again variable in different studies.

Observation regarding the clinical and biochemical correlation with HBsAg positivity is not readily available in literature. In this series an effort has been made to correlate the HBsAg positivity with clinical and biochemical profile of the cases of chronic liver diseases.

In chronic liver diseases, past history of jaundice, peritoneal tapping, oedema feet, history of immunization and/or parenteral therapy, haematemesis or malena was found to have no bearing on the positivity of HBsAg (Table 8)($p > 0.05$ in all comparisons). As one third of the HBsAg positive group denied any past history of immunization or parenteral therapy, it is likely that

there is some other route of infection of serum hepatitis by which these patients were infected. There Other workers have given along list of routes which includes kissing(Villarejos et al, 1974), human bite (Kac Quarrie et al, 1974), transplacental (Stevens et al, 1975), sharing of Razor and tooth pastes (Nostey, 1975) and blood sucking insects (Prince et al, 1972; Zebe et al, 1972). Krugman et al (1967) demonstrated that HBsAg positive serum was infective when given orally as well as parentally. HBsAg has been sought in virtually all body secretions and has been found in the urine(Tripatsis and Herst, 1971), Saliva (Ward et al, 1972), semen (Heathcote et al, 1974), bile (Adamer et al, 1971), Breast milk (Boxall et al,1974), nasal washing(Villarejos et al, 1974), Vaginal secretions(Durani and Carben, 1974), sweat (Telatar et al, 1974), cerebrospinal fluid(Dankert, et al, 1975) of subjects with HBsAg in their serum. It remains unknown whether HBsAg in body fluids, other than blood is infective. It is also possible that very often these secretions are contaminated with blood in microscopic amount, but undoubtedly, transmission of HBsAg in the absence of overt parenteral exposure takes place.

The physical findings of our study are charted in table 9 for both HBsAg positive and negative group. This shows that the presence of jaundice was significantly higher in HBsAg positive patients. However, anaemia,

collateral circulation, ascites, splenomegaly and hepatomegaly did not have any influence on HBsAg positivity. Relationship of hepatic encephalopathy with HBsAg positivity was highly statistically significant (X^2=16.91, d.f.=1, $p < 0.001$).

The biochemical values of the cases of chronic liver diseases are given in table 11. These have been analysed in relation to HBsAg positivity. Serum bilirubin was statistically significantly higher in HBsAg positive patients (t=2.82, d.f.=14, $p < 0.05$). Total serum proteins and particularly serum albumin were low and albumin-globulin ratio was almost reversed. Mean values of other biochemical tests e.g. A.S.T. and A.L.T. (Serum Aspartate aminotransferase and Serum Alanine Aminotransferase) were also significantly higher in HBsAg positive patients (* t = 2.78, d.f.= 14, $p < 0.05$ and t = 5.23, d.f.=14, $p < 0.05$ respectively).

As probably no other worker has co-rrelated in such a way, the biochemical correlation of chronic liver diseases with HBsAg positivity, it again requires further exploration in this direction.

Cases of acute viral hepatitis or chronic liver disease who developed hepatic coma, had a higher prevalence of HBsAg positivity ($p < 0.001$).

Table 17 shows the relationship of HBsAg with hepatic coma. There were 20 HBsAg positive patients.

12(60%) out of them, developed hepatic coma. On the other hand 2(6.25%) out of 30 HBsAg negative developed hepatic coma. The values were statistically significant (χ^2=4, d.f.=1, p $<$0.05). On the basis of this we can say that patient who were HBsAg positive they definitely have greater changes of hepatic coma.

TABLE 17 : Relation of HBsAg with hepatic coma.

Group	Total No. of cases	Cases who developed hepatic coma	
		No.	Percentage
HBsAg positive	20	12	60.00
HBsAg Negative	30	2	6.25

We have also studied 160 blood donors(professional-60 and voluntary-100) for HBsAg positivity. In the voluntary group we have choosen the individual from medical student, patient's relatives, nurses and paramedical staff working in the hospital or out side.

As the detection of Australia antigen in a person's serum is a specific indication of infection with humen viral hepatitis (Lancet, 1971), detection of same has become a necessity in screening of blood donors to avoid the occurrence of viral hepatitis in a recipient. For this reason, we carried out a survey of HBsAg positivity amongst sera of 100 voluntary donors. None of them was positive (prevalence 0%). One(1.66%) of the 60 professional blood donors was positive. The prevalence of Australia antigen carriers among blood

donors and in samples of normal population has been reported from any countries. Cossart (1972) reported rates varying from about 0.1 percent in USA and UK to more than 5 percent in Taiwan and other countries of south east Asia. Blumberg et al (1970) found 3 HBsAg carriers among 127 non hospitalized south Indians(2.4%). In a report from Delhi, out of 952 normal subjects, only 1 HBsAg carrier was detected and there were no positive from 30 blood donors (Sama et al, 1971).

A higher prevalence of HBsAg positive donors in the professional group has been reported from Japan (Okochi and Maurakami, 1968) and from USA(Chirubin and Prince, 1971). This situation would be some what comparable to the increased prevalence of HBsAg carriers found amongst drug addicts. (Nordenfeldt et al, 1970; Gregg, 1972). Cherubin (1971) from studies in the USA has suggested that socio-economic factors contribute to the increased prevalence in paid donors and that there is an inverse relation-ship between income and carrier state. Both of these factors contribute to the increased prevalence (3.8 percent) found in their professional donors (Hill et al, 1973).

In other studies Sama et al (1971) reported the prevalence of HBsAg in normal population to be 0.1% and Sama et al (1973) again detected HBsAg in 1.6% of voluntary blood donors from the general population,

2.73 percent of professional blood donors and 4.17 percent of donors from police training school Mehrauli. Dutta and Mohammed (1972) reported 2.65 percent HBsAg positivity amongst voluntary blood donors and 2.58 percent amongst professional blood donors. Howard et al (1970) showed widely varying incidence (0.06 to 1.47%) of HBsAg in blood donors.

Reports from other centres of the country showed the HBsAg positivity in professional blood donors at Trivandrum to be 3.4 percent (Shanmugan et al, 1978), 4.8 percent at Vellore (Hill et al, 1973), 3.9 percent at Delhi (Pastakia, 1975) and 14.4 percent at Hyderabad (Naidu and Rajyalakshmi, 1986).

In our study the prevalence of HBsAg positivity in blood donors was quite low (1.66% in professional donors and nil in voluntary). This may well be a result of the geographical variation and small number of blood donors.

CONCLUSION

1. We did not find any age or sex predeliction for HBsAg positivity in acute viral hepatitis and chronic liver diseases.
2. Latex agglutination test for HBsAg is quick, simple and easily readable by nacked eye.
3. HBsAg was present in 20(40%) of the 50 cases of liver diseases in Bundelkhand region.
4. The positivity of HBsAg in acute viral hepatitis was 35.3% (12 of 34 cases), and 50% (8 of 16 cases) in chronic liver diseases.
5. History of possible transmission by parenteral route of hepatitis 'B' virus was present in approximately three fourths of the HBsAg positive cases.
6. The prevalence rate of HBsAg positivity was higher in groups of having past history of jaundice (X^2 = 4.4, d.f. = 1, p $<$0.05), hepatic encephalopathy (X^2 = 16.25, d.f. = 1, p $<$0.001) and previous history of repeated blood transfusions (X^2 = 3.55, d.f. = 1, p $<$0.05).
7. In acute viral hepatitis HBsAg positivity was related to significantly higher serum bilirubin levels (t = 3.41, d.f. = 32, p $<$0.05). Serum aspartate aminotransferase and alanin aminotransferase levels were also higher in HBsAg positive acute viral hepatitis (t = 2.39, d.f. = 32, p $<$0.05)

and ($t = 2.04$, d.f. = 32, $p < 0.05$) and chronic liver disease ($t = 2.78$, d.f. = 14, $p < 0.05$, and $t = 5.23$, d.f. = 14, $p < 0.05$ respectively) patients.

8. None of the 100 cases of voluntary blood donors was found to be HBsAg positive. One (1.66%) out of 60 professional blood donors tested had HBsAg in his serum.

9. Patients of hepatic coma with HBsAg in their serum had worse prognosis. They also had higher incidence of coagulation and bleeding disorders.

10. The patients who were HBsAg positive in their sera showed slow recovery and had a prolonged hospital stay.

11. None of the 22 HBsAg negative patients of acute viral hepatitis died, whereas 2(16.6%) of the 12 HBsAg positive patients died.

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WORKING PROFORMA FOR HBsAg IN BUNDELKHAND REGION OF INDIA IN BLOOD DONORS (VOLUNTARY/PROFESSIONAL)

Place : Departments of Medicine and Microbiology, M.L.B. Medical College, Jhansi.

1. Name :
2. Age & Sex :
3. Religion :
4. Occupation :
5. Address :
6. Income :
7. Category : : Professional/Voluntary.
8. Previous history of blood donation : Yes/No
   If yes (i) Frequency
   (ii) Duration
9. History of blood transfusion : Yes/No
   If yes, year in which received:
10. Family history :
11. Past History :
12. Parenteral Drug History :
13. History of any addiction :
    Alcohol :
    Duration :
    Amount :
14. Examination

General Condition,	Pallor
B.P.	Oedema
Pulse Rate	Ascites
Temperature	Lymphadenopathy
Respiratory Rate	Liver
Icterus	Spleen

15. **Systemic Examination**

Respiratory

C.V.S.

C.N.S.

16. Investigations

1. Hb gm%
2. Total Serum Bilirubin : Conjugated
   : Unconjugated
3. S.G.O.T.
4. S.G.P.T.
5. Serum Alkaline Phosphatase
6. a. Total serum proteins
   b. A/G Ratio
7. HBsAg Positive/Negative
8. Others

Physician Signature
Investigator
(RMO, Med)
M.L.B. Medical College
Jhansi.

WORKING PROFORMA FOR HBsAg IN PATIENTS OF ACUTE VIRAL HEPATITIS IN BUNDELKHAND REGION OF INDIA

Place : Departments of Medicine and Microbiology,
M.L.B. Medical College,
Jhansi.

1. Name :
2. Age/Sex :
3. Religion :
4. Occupation :
5. Address :
6. Income
7. History suggestive of Acute Viral Hepatitis
   Pre icterus phase (Duration):
   Icterus phase (Duration)
   Complication(if any) :
8. History of Blood Donation
9. History of Blood Transfusion/
   If yes, year in which received :
10. Family History :
11. Past History :
12. Parenteral Drug History :
13. History of Addiction :

    Alcohol
    Duration
    Amount Yes/No

14. Examination

    General Condition Temperature
    B.P. Respiratory Rate
    Pulse Rate Icterus

Pallor Lymphadenopathy
Oedema Liver
Ascites Spleen

15. Systemic Examination

Respiratory :
C.V.S. :
C.N.S. :

16. Investigations

1. Blood Routine : TLC DLC
ESR Hb gm%

2. Blood Sugar Blood Urea

3. Bleeding time Clotting time
Prothrombin time

4. Urine : Albumin, Sugar
M/E Urobilinogen

5. Total Bilirubin
Conjugated Unconjugated
SGOT SGPT
Serum Alkaline phosphatase

6. Total Serum Proteins
A/G Ratio

7. HBsAg positive/Negative

8. Others

17. Clinical Course

Duration of Jaundice
HBsAg Positive/Negative
Complications Yes/No
Follow up

INVESTIGATOR